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(57) Abstract

The present invention relates to methods for the identification of candidate inhibitor substances that inhibit deubiquitinating activity based on the x-ray crystallographic structure of the active site of the enzyme. Changes in the properties of the enzyme are useful in identifying such substances. Also disclosed are variants of the enzyme that are useful in deubiquitinating proteins and small peptides.

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DESCRIPTION

METHODS AND COMPOSITIONS FOR A DEUBIQUITINATING ENZYME AND VARIANTS THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is generally directed to crystal protein structures, and more specifically to Ubiquitin C-terminal hydrolase, which catalyzes the removal of adducts from the C-terminus of ubiquitin.

2. Description of Related Art

Ubiquitin is a small (8.6 kDa) highly conserved protein that is best known for its role in targeting proteins for degradation by the 26S protease. Recent reviews include (Ciechanover and Schwartz, 1994; Hershko and Ciechanover, 1992; Jentsch, 1992; Wilkinson *et al.*, 1995). Ubiquitin has been implicated in numerous cellular processes, including: cell cycle control, oncoprotein degradation, receptor function, apoptosis, regulation of transcription, stress responses, maintenance of chromatin structure, DNA repair, signaling pathways, antigen presentation, and the degradation of abnormal proteins. Monomeric ubiquitin is activated by E1 (ubiquitin activating enzyme), which forms a thiolester bond with the ubiquitin C-terminus. Families of E2 (ubiquitin conjugating) and E3 (ubiquitin ligase) enzymes then catalyze ligation of the ubiquitin C-terminus to lysine side chains of acceptor proteins. Acceptor proteins can be modified with a single ubiquitin attached to one or more different lysine side chains. Alternatively, acceptor proteins can be polyubiquitinated, with a lysine side chain of the first ubiquitin conjugated to the C-terminus of the next, to form long chains attached to the target protein. Efficient targeting for degradation by the 26S protease appears to require polyubiquitination (Chau *et al.*, 1989; Gregori *et al.*, 1990). In addition to targeting proteins for degradation by the 26S protease, other roles of ubiquitination include modification of chromatin structure (Bradbury, 1992)

lysosomal targeting (Hicke and Riezman, 1996) and regulation of a kinase activity (Chen *et al.*, 1996).

In addition to isopeptide linkages to the lysine side chains of acceptor proteins, the ubiquitin C-terminus is also found attached to α-amino groups in peptide bonds, since all known ubiquitin genes encode fusion proteins in which ubiquitin is followed by a C-terminal extension (Özkaynak *et al.*, 1987). Proteolytic processing at the ubiquitin C-terminus is catalyzed by deubiquitinating enzymes (DUB). Such processing is likely to be required for several different functions, including: liberation of monomeric ubiquitin from the polyprotein precursors, release of polyubiquitin chains from the remnants of 26S protease substrates, disassembly of polyubiquitin chains to allow recycling of monomeric ubiquitin, reversal of regulatory ubiquitination, editing of inappropriately ubiquitinated proteins, and regeneration of active ubiquitin from adducts with small cellular nucleophiles (such as glutathione) that may be produced by side reactions. Additionally, several ubiquitin-like proteins that occur as fusions or conjugates have been identified, at least some of which appear to undergo a similar processing (Haas *et al.*, 1996; Matunis *et al.*, 1996; Narasimhan *et al.*, 1996; Olvera and Wool, 1993).

In light of the many different substrates, and the extensive biological consequences of ubiquitination, it is not surprising that numerous deubiquitinating enzymes have been identified. These enzymes fall into two distinct families of cysteine proteases, UBPs (ubiquitin-specific proteases) (Baker *et al.*, 1992; Tobias and Varshavsky, 1992) and UCHs (ubiquitin C-terminal hydrolases) (Pickart and Rose, 1985). Both classes of enzymes hydrolyze the peptide bond (either α- or ε-linked) at the C-terminus of ubiquitin. The UBP enzymes, 16 of which have been identified in yeast, were named for their ability to cleave large model fusion proteins at the C-terminus of ubiquitin. They vary in molecular weight from 50 kDa to 300 kDa, and exhibit a broad range of substrate specificity. Roles assigned for UBPs include cleavage of ubiquitin from the remnants of degraded protein (Papa and Hochstrasser, 1993) and disassembly of polyubiquitin chains to yield functional

monomers (Wilkinson *et al.*, 1995). They appear to function in cell fate determination (Huang *et al.*, 1995), transcriptional silencing (Henchoz *et al.*, 1996; Moazed and Johnson, 1996), and the response to cytokines (Zhu *et al.*, 1996).

The well characterized UCH enzymes are generally smaller than the UBPs, (25-28 kDa), although two larger sequences have been deposited in the GenBank database. Disruption or deletion of the one UCH gene identified in yeast confers no discernible phenotype, suggesting that the substrate specificity of UCH enzymes may overlap with the UBP enzymes (Baker *et al.*, 1992; Miller *et al.*, 1989). Biochemical studies have demonstrated that the human enzymes, UCH-L1 and UCH-L3, and the UCHs from *S. cerevisiae* and *D. melanogaster* hydrolyze ε-linked amide bonds at the C-terminus of ubiquitin (Cohen) (Roff *et al.*, 1996; Wilkinson, 1997), although most studies have focused on the hydrolysis of α-linked peptide bonds and small thiolester, ester, and amide linked adducts (Pickart and Rose, 1986; Wilkinson *et al.*, 1986). In general, most of these small adducts are good substrates, except for peptide extensions with proline immediately following the scissile bond. UCH-L3 cleaves peptide extensions of up to 20 residues from ubiquitin with high efficiency and low sequence preference, while larger folded extensions are not cleaved (Wilkinson, 1997). Similar results have been reported for the yeast UCH (Liu *et al.*, 1989; Miller *et al.*, 1989). These data suggest that the UCH enzymes may function to regenerate active ubiquitin from adducts with small nucleophiles (Pickart and Rose, 1985). The observed tissue specificity of UCH enzymes may reflect a distinct sets of substrate(s) (Wilkinson *et al.*, 1992). UCH-L1 is identical to PGP9.5, the neuronal ubiquitin C-terminal hydrolase that constitutes several percent of the total soluble protein in mammalian brain (Wilkinson *et al.*, 1989). UCH-L2 appears to be constitutively expressed in many tissues, while UCH-L3 is expressed in hematopoetic cells.

An alignment of five UCH sequences shows that only 12% of the residues are invariant (FIG. 1). Site directed mutagenesis of invariant residues on UCH-L1 implicates Cys-95 (UCH-L3 numbering) as the active site nucleophile, and His-169 as

the general base in catalysis, with an important role also played by Asp-184 (Larsen *et al.*, 1996). The UCH enzymes do not appear to share significant sequence similarity with any other protein.

In order to understand better the catalytic mechanism and substrate specificity of UCH enzymes, the inventors have determined the crystal structure of recombinant human UCH-L3 at a resolution of 1.8 Å. This structure has some similarities with the papain family of cysteine proteases, including an active site catalytic triad and oxyanion hole. A major topological difference from papain includes a 20-residue disordered loop that spans the active site. Based upon the structure, the present invention sets forth a binding orientation for ubiquitin substrates on UCH enzymes. Moreover, the invention shows that the UCH active site is normally closed and opens upon binding to substrate, and that the disordered loop may function to define the substrate specificity of UCH enzymes.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an isolated and purified amino acid sequence that encodes a deubiquitinating enzyme polypeptide UCH-L3. Preferably, a UCH-L3 peptide of the invention is a synthetic or recombinant polypeptide. More preferably, a polynucleotide of the present invention encodes a polypeptide comprising the structure of FIG. 1.

In certain embodiments, an amino acid sequence of the present invention encodes a variant UCH-L3 molecule that possesses structural differences from the native UCH-L3 protein. Such structural differences include greater stability; *i.e.* ability to resist the effects of oxidation, heat, and so forth. Moreover, such structural differences may include UCH-L3 variants that are capable of cleaving larger proteins from the ubiquitin molecule than may be accomplished by the native UCH-L3 protein.

A further advantage of the present invention includes the production of inhibitors of UCH-L3 proteins that specifically interact at the active site to reduce or eliminate UCH-L3 activity.

In yet another embodiment, the present invention contemplates a process of preparing an UCH-L3 or variant UCH-L3 comprising transfecting a cell with polynucleotide that encodes an UCH-L3 or variant UCH-L3 polypeptide to produce a transformed host cell; and maintaining the transformed host cell under biological conditions sufficient for expression of the polypeptide. The transformed host cell can be a eukaryotic cell. Alternatively, the host cell is a prokaryotic cell.

In still another embodiment, the present invention provides an antibody immunoreactive with an UCH-L3 or variant UCH-L3. Preferably, an antibody of the invention is a monoclonal antibody.

In another aspect, the present invention contemplates a process of producing an antibody immunoreactive with an UCH-L3 or variant UCH-L3 comprising the steps of (a) transfecting a recombinant host cell with a polynucleotide that encodes an UCH-L3 or variant UCH-L3; (b) culturing the host cell under conditions sufficient for expression of the polypeptide; (c) recovering the polypeptide; and (d) preparing the antibody to the polypeptide.

In yet another aspect, the present invention contemplates a process of screening substances for their ability to interact with UCH-L3 or variant UCH-L3 comprising the steps of providing an UCH-L3 or variant UCH-L3, and testing the ability of selected substances to interact with the UCH-L3 or variant UCH-L3.

In a preferred embodiment, providing an UCH-L3 or variant UCH-L3 is transfecting a host cell with a polynucleotide that encodes an UCH-L3 or variant UCH-L3 to form a transformed cell and maintaining the transformed cell under biological conditions sufficient for expression of the UCH-L3 or variant UCH-L3.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 Sequence alignment of UCH enzymes. Every tenth UCH-L3 residue is delineated with a space. Active site residues (Gln-89, Cys-95, His-169, and Asp-184 of UCH-L3) are indicated in red. Other invariant residues are indicated in orange. Secondary structural elements seen in the UCH-L3 crystal structure are indicated above the sequence, (FIG. 3). Residues that are disordered in the UCH-L3 crystal structure are indicated with broken lines. SwissProt Database entries shown are: UCH-L3, (Human; SW:P15374); UCH-L1, (Human; SW:P09936); UBL-DROME (*D. melanogaster*; SW:P35122); SCHPO, (*S. pombe*; SW:Q10171); YUH1, (*S. cerevisiae*; SW:P35127).

FIG. 2 Electron Density Map. Electron density map (blue) is shown contoured at 1.0 RMSD with the refined coordinates. Map calculation used λ1 (0.9796 Å) structure factor amplitudes in the resolution range 10-2.35 Å. The MAD phases were refined by solvent flattening and histogram shifting. The position of the selenium atom of Met-87 is apparent from the pseudo isomorphous (λ3-λ1) difference map (red), which is contoured at 30 RMSD. This figure was made with the program O (Jones *et al*, 1991)

FIG. 3 Ribbon diagram of UCH-L3. Side chains of the active site residues, Gln-89, Cys-85, His-169, and Asp-184, are shown in red and labeled Q, C, H, and D. Amino and Carboxyl termini are denoted with N and C. Residues 146 and 167, which mark the ends of the large disordered loop, are indicated. Secondary structures were defined by the program PROMOTIF (Hutchinson and Thornton,

1996). Strands are colored green and helices blue. Helix 4, which contains the active site nucleophile, Cys-95, is colored cyan. Strand 1 (29-34), strand 2 (49-57), strand 3 (168-176), strand 4 (179-183), strand 5 (191-195), strand 6 (223-229). Helix 1 (residues 13-22), helix 2 (39-42), helix 3 (60-76), helix 4 (92-110), helix 5 (118-125), helix 6 (131-140), helix 7 (201-215). Helix 4 has two kinks at residues 95 and 105 that separate the large central α-helical segment from the two short 3_{10} segments at the ends of this helix. All other helices are alpha. FIG. 3 and FIG. 5A were made with the programs MOLSCRIPT (Kraulis, 1991) and RASTER 3D (Bacon and Anderson, 1988).

FIG. 4 Comparison of UCH-L3 and Papain-like active sites. A) Active site residues of UCH-L3. Gln-89, Cys-95, His-169, and Asp-184, are shown in thick lines. A representative collection of 8 papain-like enzyme active sites are shown in thin lines following least squares overlap on the active site residue Cα atoms. The papain-like structures shown have PBD identifiers 9pap, 4pad, 1pop, 2act, 1aec, 1huc, 1csb, 1gec. Other papain-like structures used in structural comparisons in this paper are: 1the, 1cpj, 1pad, 2pad, 5pad, 6pad, 1stf, 1pip, 1ppp, 1pe6, 1ppd, 1ppn, 1ppo. Refer to the PBD for primary references to these structures, which are not included here because of space limits.

FIG. 5 Comparison of UCH-L3 with Cathepsin B. A) UCH-L3 (upper) and cathepsin B (lower) shown in a similar orientation as FIG. 3. Equivalent residues were defined by LSQMAN (Kleywegt and Jones, 1994). Pairs of C^{α} atoms were included in the overlap in their separation is less than 3.0 Å and if they form a stretch of at least 5 contiguous residues. Equivalent residues, as defined by LSQMAN, are shown in the cyan ribbon representation, and listed here: residues 32-37 of UCH-L3::residues 152-157 of cathepsin B, 48-60::166-178, 84-90::18-24, 92-106::27-40, 167-174::197-204, 182-186::217-221. B) Topology diagram of secondary structure for β-sheet and helix 4 of UCH-L3 (upper) and structurally equivalent segments of cathepsin B (lower). Secondary structural elements are colored according to their order of occurrence along the amino acid sequence; (red, orange, yellow, green, cyan,

blue, magenta). The main topological difference is for the helix, which in papain-like enzymes is the first of these secondary structural elements in the sequence, while for UCH-L3 helix 4 is found between strands 2 and 3. The long disordered loop of UCH-L3 is indicated with a dotted line.

FIG. 6 Active site clefts of papain-like enzymes and UCH-L3. Orientation is the same as for FIG. 3. A) Glycyl Endopeptidase complex with the inhibitor Benzyloxycarbonyl-L-V-G-Methylene, which occupies the S4, S3, S2 and S1 sites (O'Hara *et al.*, 1995). B) Cathepsin B with the inhibitor CA030, which occupies the S2, S1, S1' and S2' sites (Turk *et al*, 1995). Protein surfaces are colored gray/green according to curvature. Bound inhibitors are red. Active site Cys residue is yellow, other active site residues magenta. C) UCH-L3 molecular surface colored for the invariant residue of FIG. 1. Active site residues are shown in magenta, basic residues blue, acidic residue red, polar residues cyan, and hydrophobic residues green. This figure was prepared with the program GRASP (Nicholls *et al.*, 1991).

FIG. 7 Proposed orientation of UCH-L3/Ubiquitin binding. This view is approximately perpendicular (from the left) of FIG. 3. Crystal structure of UCH-L3 is shown with β-strands green, helix-4 cyan, and other structure yellow. The glycyl endopeptidase and cathepsin B S and S' site inhibitors of FIG. 6A and FIG. 6B are shown in red and magenta respectively after least squares overlap of the papain-like enzyme complexes on the UCH-L3 crystal structure. The structure of ubiquitin (Vijay-Kumar *et al.*, 1987), shown in gray, has been positioned with the basic face adjacent to UCH-L3, the C-terminal carboxylate adjacent to UCH-L3 Cys-95, and with the flexible C-terminal residues following the path of the Glycyl Endopeptidase S site inhibitor.

FIG. 8 The active site cleft of UCH-L3 is blocked. Stereoview of the UCH-L3 active site in approximately the same orientation as FIG. 3. The active site residues Gln-89, Cys-95, His-169, and Asp-184, are labeled with Q, C, H, and D, respectively. UCH-L3 residues Leu-9, Glu-10, Ala-11, and Ser-92 are labeled.

UCH-L3 is colored cyan, with the two segments proposed to move upon binding substrate colored green (residues 9-12; 90-94). The S4-S1 site inhibitor of Glycyl Endopeptidase (FIG. 6A) is shown in red after superposition on the UCH active site residue C^{α} atoms.

FIG. 9 Possible orientations of the UCH-L3 disordered loop. The crystal structure of UCH-L3 is shown in the same color representation and orientation as FIG. 7. The docked ubiquitin molecule has been moved slightly away from the UCH-L3 for clarity. Residues that follow ubiquitin in an α-linked substrate adduct have been included in dark gray color. Three possible classes of conformation are shown in magenta, blue, and red, for the disordered loop (residues 147-166) with respect to the substrate.

FIG. 10 Relative rates of hydrolysis of ubiquitin derivatives by UCH isozymes. The rates of hydrolysis were measured by HPLC according to Wilkinson et al. (1986). The brackets [] surround the leaving group. The rates shown are obtained with 15 μM substrates (~ 20 times K_m) and are given as the ratio of rates for the indicated substrate vs. that for ubiquitin ethyl ester. The error bars represent the standard error of the mean (See Table 1 for absolute rates). Note the log scale.

FIG. 11 UbCEP52 is a substrate for UCH-L3. Each lane contains 10 μg of substrate and 1 μg of enzyme. The time of digestion is given in minutes. **A**: SDS-PAGE of the reaction time course, protein detected by Coomassie Blue staining. **B**: Immunoblot of a duplicate gel, probed with rabbit antisera to human CEP52. The unmarked band is a minor contaminant.

FIG. 12 Nucleic acid inhibits the processing of UbCEP52 by UCH-L3. Nucleic acid was added at a concentration of 0.05 mg/mL, and incubated for ten minutes with the substrate before enzyme was added to start the reaction. Addition of dsDNA to UbOEt had no effect on the rate of ester hydrolysis (triangles). The rate of hydrolysis of UbCEP52 is only a few-fold slower (+). Addition of RNAse A slightly

increased the rate of hydrolysis of UbCEP52 (x). Single stranded DNA had little effect (solid circles), while either *E. coli* RNA (solid squares), a plasmid DNA (open circles), or a double-stranded 42 bp DNA (open squares) significantly inhibited.

FIG. 13 Co-translational processing of the proubiquitin (left panel) and UbCEP80 (right panel) gene products by UCH-L1 and UCH-L3. The bacterial host BL21(DE3) was co-transformed with a plasmid encoding the substrates and the Amp^r gene product and a second vector encoding the indicated enzyme and Kan^r gene product. Protein production was induced with IPTG for three hours and whole cell lysates were subjected to SDS-PAGE and immunoblotting with anti-ubiquitin (left panel) or anti-CEP80 (right panel) antibodies.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present inventors have determined the UCH-L3 crystal structure in atomic detail, which provides the basis for altering the active site of the protein. UCH-L3 belongs to a family of UCH (ubiquitin C-terminal hydrolase) enzymes that all catalyze the removal of adducts from the C-terminus of the small protein ubiquitin. Because the similarity in amino acid sequences indicates that all of the UCH enzymes will have the same three dimensional structure, and because UCH-L3 is the first UCH for which a structure is known, the present invention is applicable to all UCH enzymes.

UCH-L3 has a core catalytic structure that strongly resembles cathepsin B, a papain-like protease. The active site groove is occluded by two loops, and it is postulated that a substrate-induced conformational change is required to clear the cleft and allow access to the active-site cysteine. Thus, only ubiquitin derivatives are substrates because only they can form the extensive interactions with the S′ site required to trigger the necessary conformational change generating the active conformation of the enzyme.

Specificity for P′ residues must be determined by the residues lining the corresponding S′ sites on the UCH enzymes. The sequence of these proteins varies widely in several areas, including a region just N-terminal to the active site histidine. This sequence is disordered in the UCH-L3 structure, but may be positioned to form a significant contact region with the P′ residues of substrates. Thus, it is likely that this hypervariable region is important in determining substrate selectivity and the somewhat shorter loop near the active site cysteine in UCH-L1 restricts the possible substrates by conferring a narrower or more restricted active site cleft.

UCH enzymes have potential in the commercial production of peptides (and possibly proteins), that are initially expressed as ubiquitin fusions from which the ubiquitin is later cleaved by UCH activity. UCH-L3 is already used in this way for the production of peptides. *See*, for example, U.S. Patent No. 5,620,923. The utility of this process could be enhanced by the availability of a UCH enzyme that possess greater thermal and/or chemical stability.

Knowledge of the UCH-L3 structure can be used to design variants with enhanced properties such as increased stability. This work can be performed by inspection of the UCH-L3 structure on a graphics workstation, by computer manipulation of UCH-L3 coordinates, and calculations such as energy minimization. Variants whose potential properties are initially predicted in light of the UCH-L3 structure can be produced by the usual techniques of molecular biology.

Enhanced stability might result from increasing the number of salt bridge or hydrogen bonding interactions, improving the packing of hydrophobic groups, or by adding disulfide bonds. Chemical stability might be improved by replacement of chemically labile groups with more stable amino acid residues. For example, buried methionine residues might be replaced with the more inert leucine amino acid residue. Cysteine residues might also be replaced, for example with alanine or serine side chains.

The present invention provides teaching to replace an active site residue, such as cysteine-95, with another amino acid residue to produce a more stable enzyme that uses a serine protease mechanism rather than the cysteine protease mechanism of wild type UCH enzymes. For example, the present invention provides guidance to make other changes in the enzyme structure, such changing aspartic acid to asparagine, to alter the specificity or stability of the enzyme. All such approaches to produce a more stable enzyme will be assisted by knowledge of the UCH-L3 structure.

Because ubiquitin chemistry is of fundamental importance to cellular metabolism, it may be possible to design therapeutic agents that function through modification of UCH activities. For example, a specific UCH inhibitor may increase (or reduce) the rate at which a protein(s) is degraded by the 26S protease. Because some proteins that function in proliferation are normally turned over by ubiquitin-mediated degradation, UCH inhibitors may have utility in the treatment of cancers. Another possible utility is in the treatment of wasting diseases which are thought to result from excessive ubiquitin-mediated proteolysis. UCH inhibitors may also find utility in the treatment of neurodegenerative diseases, since the UCH-L1 isozyme is highly abundant in neuronal tissue, and these diseases are characterized by deposits that are rich in ubiquitin conjugates (*i.e.* UCH substrates).

Ubiquitin C-terminal hydrolases catalyze the removal of adducts from the C-terminus of ubiquitin. The present inventors have determined the crystal structure of the recombinant human ubiquitin C-terminal hydrolase, UCH-L3, by X-ray crystallography at 1.8 Å resolution. The structure is comprised of a central antiparallel β-sheet flanked on both sides by α-helices. The β-sheet and one of the helices resemble the well known papain-like cysteine proteases, with the greatest similarity to cathepsin B. This similarity includes the UCH-L3 active site catalytic triad of Cys-95, His-169 and Asp-184, and the oxyanion hole residue Gln-89. Papain and UCH-L3 differ, however, in strand and helix connectivity, which in the UCH-L3 structure includes a disordered 20-residue loop (res 147-166) that is positioned over the active site and may function in the definition of substrate specificity. Based upon

analogy with inhibitor complexes of the papain-like enzymes, the inventors set forth the following mechanism to describe the binding of ubiquitin to UCH-L3. The UCH-L3 active site cleft appears to be masked in the unliganded structure by two different segments of the enzyme (res 9-12 and 90-94), thus implying a conformational change upon substrate binding and suggesting a mechanism to limit non-specific hydrolysis.

Crystallization

The recombinant human UCH-L3 used in these studies was purified as described (Larsen *et al.*, 1996). The protein solution used in crystallization trials was 12 mg/mL UCH-L3 in 50 mM Tris Hcl, pH 7.6, 15 mM BME, 1 mM EDTA. This solution was stored in aliquots at -70°C. Crystallization was performed at 4°C in sitting drops. The reservoir solution was 26% (w/w) PEG 4000, 200 mM sodium acetate, 100 mM Pipes pH 6.7, and 10 mM DTT. The drop solution was 3 μL of protein solution mixed with 3 μL of reservoir solution. These conditions produced crystalline aggregates after 4-5 days.

Single crystals were obtained by microseeding. One of the initial aggregates was ground up with a needle, and the needle streaked through a drop that was identical to the conditions described above, but which had equilibrated for 3-5 days. Small single crystals appeared after several days.

Large crystals were obtained by macroseeding. Using a rayon loop, a small single crystal was transferred into reservoir solution, allowed to wash for several minutes, and then transferred into another drop that has been equilibrated for 3-5 days. The same reservoir and drop condition used to obtain the initial aggregates were also used for the subsequent micro and macroseeding. The crystals attain their maximum size in 5-10 days following macroseeding. Typical crystal dimensions are 0.3 mm × 0.3 mm × 0.6 mm.

For generation of selenomethionine-substituted UCH-L3 (SeUCH-L3), the *gal-,met-* auxotroph B834(DE3) of the BL21 strain (Studier and Moffatt, 1986) harboring pRSL3 (Larsen *et al.*, 1996) was grown on LB agar as colonies. A single colony was inoculated into 50 mL LB media and grown overnight, followed by dilution into 6 liters of modified M9 media. Solutions O, P, S, and V (Weber *et al.*, 1992), uracil (Final concentration of 1 mM), and selenomethionine (final concentration of 50 μg/l) were sterile filtered and added to M9 media.

At an OD_{600}nm of 0.6, the cells were induced with 0.5 mM IPTG for three hours before harvesting by centrifugation. Purification of SeUCH-L3 was the same as for wild type. Ion electrospray mass spectrometry showed an incorporation of >98% Se at each Met codon. SeUCH-L3 and wild type UCH-L3 have comparable specific activities. SeUCH-L3 crystals were grown under the same conditions as native protein, although in this case the seeding steps proved unnecessary and growth time from initial set up was 5-10 days.

Data Collection and Processing

The native and SeUCH-L3 crystals are isomorphous; space group $P2_12_12_1$, cell dimensions: a=48.6 Å, b=60.8 Å, c=81.4 Å. There is one molecule in the asymmetric unit, and the Matthew's parameter, V_m, is 2.37 $Å^3Da^{-1}$, which corresponds to a solvent content of 48% (Matthews, 1968).

All data were collected at 100K. Prior to cryocooling the crystals were transferred to the reservoir solution, and then to a series of solutions that were identical except for 2% increments in glycerol concentration up to a final concentration of 18% glycerol. The cryoprotected crystals were suspended in a rayon loop and cooled by plunging into liquid nitrogen.

Multiwavelength data were collected from a single SeUCH-L3 crystal on a MAR imaging plate detector at beamline X12C of the National Synchrotron Light

Source, Brookhaven National Laboratory. The three wavelengths collected were selected from the fluorescence spectrum; λ1 (0.9796 Å) was chosen as the inflection, or rise, corresponding to the minimum value of f'; λ2 (0.9793 Å) was taken as the peak, corresponding to the maximum in f"; λ3 (0.9300 Å) was chosen for the remote wavelength, corresponding to the maximum in f'. Data from each wavelength were indexed and integrated independently, and data from all three wavelengths were scaled together from 6.0 Å to 2.2 Å. The resulting scale factors were then applied separately to each individual wavelength for data from 30 Å to 2.35 Å. Data from a native crystal were collected to 1.8 Å resolution on a MAR imaging plate detector at beamline 7-1 of the Stanford Synchrotron Radiation Laboratory, Palo Alto. All data were processed with the programs DENZO and SCALEPAK (Otwinowski, 1993). See Table 1 for data statistics.

Table 1. Data Processing Statistics

	Native	**λ1**	**λ2**	**λ3**
	SSRL[a]	NSLS[a]	NSLS[a]	NSLS[a]
Wavelength (Å)	1.080	0.9796	0.9793	0.930
Resolution limit (Å)	1.80	2.35	2.35	2.35
High resol. shell (Å)	(1.83-1.80)	(2.43-2.35)	(2.43-2.35)	(2.43-2.35)
#Unique reflections	23334	11282	11217	11108
Completeness (%)	98 (93)	96 (86)	90 (84)	89 (91)
$\langle I/\sigma_{(I)}\rangle$	20 (5)	15 (5)	15 (5)	15 (6)
Redundancy[b]	4.5 (3)	2 (1)	2 (1)	2 (1.5)
R sym (%)[c]	4.0 (19)	4.9 (13.2)	4.4 (12.1)	4.8 (13.7)
Mosaicity (°)	1.18	0.42	0.42	0.42

[a] Data were collected on MAR imaging plate detectors on beamline 7-1 at the Stanford Synchrotron Radiation Laboratory (SSRL) or beamline X12C of the National Synchrotron Light Source (NSLS).

[b] Redundancy is defined as the ratio of observed/unique structure factor amplitudes.

[c] R sym = $100 * \Sigma_{hkl}\Sigma i|I_i-\langle I\rangle| = \Sigma\langle I\rangle$

Data were processed with DENZO and SCALEPACK (Otwinowski, 1993).

Structure Determination and Refinement

Crystallographic computing was performed using programs from the CCP4 suite (CCP4, 1994), unless otherwise stated. Of the seven methionine residues in UCH-L3, all except the amino terminal Met are ordered. The six selenium sites were identified from difference Patterson and Fourier functions using the program XtalView (McRee, 1992). Selenium parameters were refined in MLPHARE (Otwinowski, 1991), treating λ1 as the native data of a conventional multiple isomorphous phase determination (Ramakrishnan and Biou, 1997). The mean figure of merit calculated by MLPHARE was 0.42.

Phases computed with MLPHARE were refined by solvent flattening and histogram shifting with the program DM (Cowtan, 1994) to a mean figure of merit of 0.77. The resulting electron density map was readily interpretable for the majority of the UCH-L3 sequence, see FIG. 2. Rounds of refinement with XPLOR (Brünger, 1992b) were interspersed with mode building (Jones *et al.*, 1991). λ1 amplitudes from 10.0 Å to 2.35 Å resolution were used in the refinement, with phase restraints also applied. At this stage the Rvalue against 10.0 Å to 2.35 Å data was 24.3% and the free Rvalue was 30.4% (Brünger, 1992a). No sigma cuts were applied to refinement or Rvalue calculations.

Refinement was continued against 6.0 Å to 1.8 Å data collected from a native crystal (see Table 2). Because of a slight deviation from true isomorphism between the native and SeUCH-L3 crystals, phase restrains were not employed for the high resolution refinement. The final model includes 121 water molecules and 205 of the total 230 UCH-L3 residues. The current Rvalue is 23.0% and the free Rvalue is 28.6%. The first four residues at the amino-terminus are disordered, as are residues 147-166 and 218. The model has good stereochemistry as judged by PROCHECK (Laskowski *et al.*, 1993).

UCH-L3 COORDINATES

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ATOM  195  CG   LEU  23  28.621 25.216  54.126  1.00  27.13 AAAA
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ATOM  197  CD2  LEU  23  28.650 24.292  55.360  1.00  22.91 AAAA
ATOM  198  C    LEU  23  25.046 26.722  54.690  1.00  21.30 AAAA
ATOM  199  O    LEU  23  25.134 27.307  55.770  1.00  19.91 AAAA
ATOM  200  N    GLY  24  24.102 27.009  53.792  1.00  22.47 AAAA
ATOM  201  H    GLY  24  24.162 26.600  52.902  1.00   0.00 AAAA
ATOM  202  CA   GLY  24  22.979 27.880  54.123  1.00  18.64 AAAA
ATOM  203  C    GLY  24  23.062 29.291  53.583  1.00  20.19 AAAA
ATOM  204  O    GLY  24  22.359 30.187  54.058  1.00  21.11 AAAA
ATOM  205  N    LEU  25  23.917 29.497  52.582  1.00  21.94 AAAA
ATOM  206  H    LEU  25  24.454 28.744  52.264  1.00   0.00 AAAA
ATOM  207  CA   LEU  25  24.070 30.802  51.936  1.00  22.38 AAAA
ATOM  208  CB   LEU  25  25.525 31.053  51.514  1.00  21.52 AAAA
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ATOM	209	CG	LEU	25	26.611	31.599	52.432	1.00	24.11	AAAA
ATOM	210	CD1	LEU	25	26.183	32.924	52.976	1.00	26.31	AAAA
ATOM	211	CD2	LEU	25	26.911	30.589	53.536	1.00	25.31	AAAA
ATOM	212	C	LEU	25	23.218	30.870	50.670	1.00	23.83	AAAA
ATOM	213	O	LEU	25	23.382	30.069	49.744	1.00	22.04	AAAA
ATOM	214	N	HIS	26	22.437	31.935	50.568	1.00	27.33	AAAA
ATOM	215	H	HIS	26	22.373	32.444	51.402	1.00	0.00	AAAA
ATOM	216	CA	HIS	26	21.827	32.302	49.297	1.00	30.72	AAAA
ATOM	217	CB	HIS	26	20.858	33.461	49.519	1.00	35.03	AAAA
ATOM	218	CG	HIS	26	19.725	33.126	50.440	1.00	39.71	AAAA
ATOM	219	CD2	HIS	26	19.605	33.253	51.781	1.00	38.99	AAAA
ATOM	220	ND1	HIS	26	18.546	32.562	49.999	1.00	40.72	AAAA
ATOM	221	HD1	HIS	26	18.332	32.241	49.089	1.00	0.00	AAAA
ATOM	222	CE1	HIS	26	17.743	32.369	51.032	1.00	39.92	AAAA
ATOM	223	NE2	HIS	26	18.359	32.785	52.123	1.00	39.81	AAAA
ATOM	224	HE2	HIS	26	18.134	32.577	53.054	1.00	0.00	AAAA
ATOM	225	C	HIS	26	22.910	32.705	48.286	1.00	30.28	AAAA
ATOM	226	O	HIS	26	23.899	33.337	48.655	1.00	32.24	AAAA
ATOM	227	N	PRO	27	22.681	32.456	46.989	1.00	28.45	AAAA
ATOM	228	CD	PRO	27	21.500	31.803	46.395	1.00	30.37	AAAA
ATOM	229	CA	PRO	27	23.753	32.629	46.003	1.00	24.28	AAAA
ATOM	230	CB	PRO	27	23.367	31.657	44.904	1.00	27.20	AAAA
ATOM	231	CG	PRO	27	21.850	31.731	44.917	1.00	31.25	AAAA
ATOM	232	C	PRO	27	23.856	34.044	45.465	1.00	23.59	AAAA
ATOM	233	O	PRO	27	23.941	34.235	44.267	1.00	28.42	AAAA
ATOM	234	N	ASN	28	23.971	35.021	46.349	1.00	21.98	AAAA
ATOM	235	H	ASN	28	24.029	34.719	47.277	1.00	0.00	AAAA
ATOM	236	CA	ASN	28	24.197	36.393	45.911	1.00	25.40	AAAA
ATOM	237	CB	ASN	28	23.630	37.402	46.913	1.00	29.65	AAAA
ATOM	238	CG	ASN	28	23.848	36.989	48.341	1.00	36.22	AAAA
ATOM	239	OD1	ASN	28	24.378	37.743	49.162	1.00	39.03	AAAA
ATOM	240	ND2	ASN	28	23.289	35.853	48.689	1.00	39.95	AAAA
ATOM	241	HD21	ASN	28	23.560	35.562	49.587	1.00	0.00	AAAA
ATOM	242	HD22	ASN	28	22.654	35.468	48.059	1.00	0.00	AAAA
ATOM	243	C	ASN	28	25.676	36.670	45.714	1.00	24.12	AAAA
ATOM	244	O	ASN	28	26.063	37.766	45.316	1.00	25.78	AAAA
ATOM	245	N	TRP	29	26.490	35.759	46.237	1.00	22.87	AAAA
ATOM	246	H	TRP	29	26.120	35.099	46.851	1.00	0.00	AAAA
ATOM	247	CA	TRP	29	27.926	35.739	45.966	1.00	21.65	AAAA
ATOM	248	CB	TRP	29	28.737	36.004	47.245	1.00	19.38	AAAA
ATOM	249	CG	TRP	29	28.640	37.388	47.766	1.00	18.45	AAAA
ATOM	250	CD2	TRP	29	29.441	38.519	47.389	1.00	18.42	AAAA
ATOM	251	CE2	TRP	29	28.946	39.636	48.101	1.00	19.19	AAAA

ATOM	252	CE3	TRP	29	30.518	38.706	46.510	1.00	19.40 AAAA
ATOM	253	CD1	TRP	29	27.736	37.846	48.672	1.00	19.49 AAAA
ATOM	254	NE1	TRP	29	27.909	39.191	48.881	1.00	19.40 AAAA
ATOM	255	HE1	TRP	29	27.325	39.692	49.489	1.00	0.00 AAAA
ATOM	256	CZ2	TRP	29	29.487	40.924	47.961	1.00	19.87 AAAA
ATOM	257	CZ3	TRP	29	31.057	39.993	46.367	1.00	18.65 AAAA
ATOM	258	CH2	TRP	29	30.539	41.079	47.090	1.00	18.95 AAAA
ATOM	259	C	TRP	29	28.233	34.352	45.446	1.00	19.35 AAAA
ATOM	260	O	TRP	29	27.682	33.369	45.953	1.00	21.87 AAAA
ATOM	261	N	GLN	30	29.000	34.275	44.357	1.00	19.97 AAAA
ATOM	262	H	GLN	30	29.276	35.096	43.900	1.00	0.00 AAAA
ATOM	263	CA	GLN	30	29.401	32.984	43.783	1.00	19.00 AAAA
ATOM	264	CB	GLN	30	28.543	32.671	42.552	1.00	20.67 AAAA
ATOM	265	CG	GLN	30	27.042	32.621	42.844	1.00	21.49 AAAA
ATOM	266	CD	GLN	30	26.632	31.302	43.408	1.00	20.18 AAAA
ATOM	267	OE1	GLN	30	26.459	30.349	42.671	1.00	23.21 AAAA
ATOM	268	NE2	GLN	30	26.636	31.188	44.725	1.00	22.75 AAAA
ATOM	269	HE21	GLN	30	26.874	31.932	45.294	1.00	0.00 AAAA
ATOM	270	HE22	GLN	30	26.395	30.274	44.998	1.00	0.00 AAAA
ATOM	271	C	GLN	30	30.876	32.995	43.383	1.00	18.13 AAAA
ATOM	272	O	GLN	30	31.378	34.008	42.896	1.00	19.67 AAAA
ATOM	273	N	PHE	31	31.575	31.892	43.615	1.00	18.15 AAAA
ATOM	274	H	PHE	31	31.160	31.192	44.148	1.00	0.00 AAAA
ATOM	275	CA	PHE	31	32.952	31.764	43.136	1.00	18.12 AAAA
ATOM	276	CB	PHE	31	33.651	30.576	43.791	1.00	18.21 AAAA
ATOM	277	CG	PHE	31	34.089	30.841	45.211	1.00	21.78 AAAA
ATOM	278	CD1	PHE	31	33.322	30.396	46.288	1.00	18.71 AAAA
ATOM	279	CD2	PHE	31	35.263	31.563	45.476	1.00	20.73 AAAA
ATOM	280	CE1	PHE	31	33.719	30.657	47.608	1.00	18.40 AAAA
ATOM	281	CE2	PHE	31	35.663	31.826	46.789	1.00	17.15 AAAA
ATOM	282	CZ	PHE	31	34.893	31.374	47.856	1.00	16.61 AAAA
ATOM	283	C	PHE	31	33.023	31.606	41.631	1.00	20.44 AAAA
ATOM	284	O	PHE	31	32.214	30.891	41.025	1.00	21.52 AAAA
ATOM	285	N	VAL	32	34.029	32.232	41.034	1.00	21.92 AAAA
ATOM	286	H	VAL	32	34.545	32.853	41.588	1.00	0.00 AAAA
ATOM	287	CA	VAL	32	34.349	32.045	39.616	1.00	20.67 AAAA
ATOM	288	CB	VAL	32	34.004	33.332	38.780	1.00	20.87 AAAA
ATOM	289	CG1	VAL	32	32.508	33.586	38.787	1.00	23.52 AAAA
ATOM	290	CG2	VAL	32	34.702	34.563	39.345	1.00	18.97 AAAA
ATOM	291	C	VAL	32	35.847	31.736	39.494	1.00	21.19 AAAA
ATOM	292	O	VAL	32	36.619	32.038	40.405	1.00	19.52 AAAA
ATOM	293	N	ASP	33	36.251	31.088	38.407	1.00	20.92 AAAA
ATOM	294	H	ASP	33	35.575	30.751	37.793	1.00	0.00 AAAA

ATOM	295	CA	ASP	33	37.677	30.936	38.103	1.00	24.10 AAAA
ATOM	296	CB	ASP	33	37.877	30.087	36.867	1.00	23.40 AAAA
ATOM	297	CG	ASP	33	37.547	28.662	37.101	1.00	28.62 AAAA
ATOM	298	OD1	ASP	33	38.155	28.078	38.023	1.00	31.38 AAAA
ATOM	299	OD2	ASP	33	36.637	28.146	36.415	1.00	31.16 AAAA
ATOM	300	C	ASP	33	38.324	32.278	37.849	1.00	24.54 AAAA
ATOM	301	O	ASP	33	37.694	33.163	37.268	1.00	25.36 AAAA
ATOM	302	N	VAL	34	39.547	32.465	38.333	1.00	23.21 AAAA
ATOM	303	H	VAL	34	39.885	31.900	39.060	1.00	0.00 AAAA
ATOM	304	CA	VAL	34	40.379	33.527	37.787	1.00	26.15 AAAA
ATOM	305	CB	VAL	34	41.176	34.265	38.881	1.00	25.11 AAAA
ATOM	306	CG1	VAL	34	42.030	35.353	38.243	1.00	24.72 AAAA
ATOM	307	CG2	VAL	34	40.216	34.871	39.916	1.00	23.17 AAAA
ATOM	308	C	VAL	34	41.324	32.869	36.769	1.00	26.65 AAAA
ATOM	309	O	VAL	34	41.990	31.884	37.080	1.00	27.57 AAAA
ATOM	310	N	TYR	35	41.149	33.214	35.500	1.00	26.68 AAAA
ATOM	311	H	TYR	35	40.563	33.967	35.283	1.00	0.00 AAAA
ATOM	312	CA	TYR	35	41.782	32.450	34.430	1.00	26.52 AAAA
ATOM	313	CB	TYR	35	41.012	32.649	33.116	1.00	25.88 AAAA
ATOM	314	CG	TYR	35	39.736	31.831	33.066	1.00	22.69 AAAA
ATOM	315	CD1	TYR	35	38.494	32.409	33.344	1.00	22.08 AAAA
ATOM	316	CE1	TYR	35	37.348	31.628	33.440	1.00	18.63 AAAA
ATOM	317	CD2	TYR	35	39.791	30.455	32.872	1.00	24.37 AAAA
ATOM	318	CE2	TYR	35	38.657	29.672	32.964	1.00	23.29 AAAA
ATOM	319	CZ	TYR	35	37.442	30.264	33.251	1.00	21.25 AAAA
ATOM	320	OH	TYR	35	36.321	29.472	33.331	1.00	22.79 AAAA
ATOM	321	HH	TYR	35	35.794	29.791	34.083	1.00	0.00 AAAA
ATOM	322	C	TYR	35	43.262	32.795	34.266	1.00	28.14 AAAA
ATOM	323	O	TYR	35	44.064	31.950	33.869	1.00	31.16 AAAA
ATOM	324	N	GLY	36	43.615	34.023	34.626	1.00	30.78 AAAA
ATOM	325	H	GLY	36	42.903	34.661	34.827	1.00	0.00 AAAA
ATOM	326	CA	GLY	36	45.013	34.404	34.757	1.00	35.11 AAAA
ATOM	327	C	GLY	36	45.130	35.712	35.520	1.00	37.27 AAAA
ATOM	328	O	GLY	36	44.114	36.294	35.889	1.00	38.81 AAAA
ATOM	329	N	MET	37	46.356	36.177	35.756	1.00	41.81 AAAA
ATOM	330	H	MET	37	47.091	35.568	35.539	1.00	0.00 AAAA
ATOM	331	CA	MET	37	46.590	37.477	36.400	1.00	44.35 AAAA
ATOM	332	CB	MET	37	47.883	37.457	37.231	1.00	48.91 AAAA
ATOM	333	CG	MET	37	48.285	36.101	37.820	1.00	53.53 AAAA
ATOM	334	SD	MET	37	47.107	35.436	39.011	1.00	65.33 AAAA
ATOM	335	CE	MET	37	48.083	34.161	39.821	1.00	58.45 AAAA
ATOM	336	C	MET	37	46.678	38.598	35.356	1.00	45.24 AAAA
ATOM	337	O	MET	37	46.695	39.778	35.694	1.00	48.93 AAAA

ATOM	338	N	ASP	38	46.841	38.214	34.095	1.00	44.40 AAAA
ATOM	339	H	ASP	38	47.007	37.262	33.943	1.00	0.00 AAAA
ATOM	340	CA	ASP	38	46.750	39.127	32.958	1.00	44.85 AAAA
ATOM	341	CB	ASP	38	46.718	38.296	31.668	1.00	54.39 AAAA
ATOM	342	CG	ASP	38	47.302	39.024	30.461	1.00	61.36 AAAA
ATOM	343	OD1	ASP	38	47.368	40.273	30.451	1.00	65.74 AAAA
ATOM	344	OD2	ASP	38	47.647	38.328	29.478	1.00	67.49 AAAA
ATOM	345	C	ASP	38	45.477	39.980	33.038	1.00	42.01 AAAA
ATOM	346	O	ASP	38	44.382	39.453	33.209	1.00	38.13 AAAA
ATOM	347	N	PRO	39	45.592	41.284	32.746	1.00	42.09 AAAA
ATOM	348	CD	PRO	39	46.871	42.020	32.774	1.00	43.41 AAAA
ATOM	349	CA	PRO	39	44.441	42.193	32.631	1.00	40.63 AAAA
ATOM	350	CB	PRO	39	45.046	43.461	32.042	1.00	40.54 AAAA
ATOM	351	CG	PRO	39	46.464	43.449	32.509	1.00	42.45 AAAA
ATOM	352	C	PRO	39	43.315	41.673	31.744	1.00	40.33 AAAA
ATOM	353	O	PRO	39	42.146	41.990	31.959	1.00	40.35 AAAA
ATOM	354	N	GLU	40	43.683	40.979	30.675	1.00	39.70 AAAA
ATOM	355	H	GLU	40	44.637	40.883	30.494	1.00	0.00 AAAA
ATOM	356	CA	GLU	40	42.695	40.457	29.736	1.00	39.58 AAAA
ATOM	357	CB	GLU	40	43.391	39.907	28.487	1.00	38.40 AAAA
ATOM	358	CG	GLU	40	43.806	41.003	27.512	1.00	39.64 AAAA
ATOM	359	CD	GLU	40	44.733	40.531	26.388	1.00	39.81 AAAA
ATOM	360	OE1	GLU	40	45.443	39.501	26.546	1.00	35.20 AAAA
ATOM	361	OE2	GLU	40	44.802	41.261	25.372	1.00	37.81 AAAA
ATOM	362	C	GLU	40	41.823	39.381	30.377	1.00	38.35 AAAA
ATOM	363	O	GLU	40	40.616	39.318	30.134	1.00	39.28 AAAA
ATOM	364	N	LEU	41	42.426	38.578	31.248	1.00	36.49 AAAA
ATOM	365	H	LEU	41	43.381	38.714	31.409	1.00	0.00 AAAA
ATOM	366	CA	LEU	41	41.691	37.538	31.966	1.00	35.44 AAAA
ATOM	367	CB	LEU	41	42.567	36.300	32.135	1.00	34.47 AAAA
ATOM	368	CG	LEU	41	42.961	35.650	30.805	1.00	33.48 AAAA
ATOM	369	CD1	LEU	41	44.058	34.633	31.026	1.00	34.40 AAAA
ATOM	370	CD2	LEU	41	41.754	35.002	30.181	1.00	26.42 AAAA
ATOM	371	C	LEU	41	41.161	38.015	33.321	1.00	34.73 AAAA
ATOM	372	O	LEU	41	40.068	37.623	33.735	1.00	35.09 AAAA
ATOM	373	N	LEU	42	41.834	38.995	33.918	1.00	33.06 AAAA
ATOM	374	H	LEU	42	42.629	39.346	33.483	1.00	0.00 AAAA
ATOM	375	CA	LEU	42	41.370	39.565	35.177	1.00	31.32 AAAA
ATOM	376	CB	LEU	42	42.426	40.495	35.778	1.00	31.53 AAAA
ATOM	377	CG	LEU	42	43.141	40.129	37.083	1.00	34.19 AAAA
ATOM	378	CD1	LEU	42	43.441	41.416	37.830	1.00	37.43 AAAA
ATOM	379	CD2	LEU	42	42.304	39.211	37.950	1.00	33.17 AAAA
ATOM	380	C	LEU	42	40.074	40.344	34.983	1.00	31.86 AAAA

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ATOM  381  O   LEU  42  39.146 40.232  35.782  1.00  31.41 AAAA
ATOM  382  N   SER  43  39.993 41.114  33.905  1.00  32.09 AAAA
ATOM  383  H   SER  43  40.722 41.122  33.244  1.00   0.00 AAAA
ATOM  384  CA  SER  43  38.809 41.942  33.660  1.00  30.91 AAAA
ATOM  385  CB  SER  43  39.067 42.895  32.488  1.00  30.06 AAAA
ATOM  386  OG  SER  43  39.651 42.207  31.402  1.00  33.39 AAAA
ATOM  387  HG  SER  43  40.500 42.602  31.162  1.00   0.00 AAAA
ATOM  388  C   SER  43  37.540 41.111  33.401  1.00  27.31 AAAA
ATOM  389  O   SER  43  36.430 41.614  33.510  1.00  27.97 AAAA
ATOM  390  N   MET  44  37.709 39.819  33.160  1.00  24.99 AAAA
ATOM  391  H   MET  44  38.618 39.473  33.038  1.00   0.00 AAAA
ATOM  392  CA  MET  44  36.570 38.918  33.035  1.00  27.37 AAAA
ATOM  393  CB  MET  44  37.007 37.600  32.420  1.00  29.97 AAAA
ATOM  394  CG  MET  44  37.265 37.623  30.948  1.00  30.45 AAAA
ATOM  395  SD  MET  44  38.047 36.072  30.555  1.00  40.12 AAAA
ATOM  396  CE  MET  44  36.647 34.951  30.644  1.00  38.79 AAAA
ATOM  397  C   MET  44  35.838 38.595  34.351  1.00  30.11 AAAA
ATOM  398  O   MET  44  34.770 37.981  34.337  1.00  31.53 AAAA
ATOM  399  N   VAL  45  36.484 38.830  35.484  1.00  29.44 AAAA
ATOM  400  H   VAL  45  37.345 39.290  35.448  1.00   0.00 AAAA
ATOM  401  CA  VAL  45  35.893 38.445  36.763  1.00  26.30 AAAA
ATOM  402  CB  VAL  45  36.965 38.411  37.875  1.00  24.98 AAAA
ATOM  403  CG1 VAL  45  36.338 38.051  39.227  1.00  21.57 AAAA
ATOM  404  CG2 VAL  45  38.043 37.412  37.503  1.00  23.63 AAAA
ATOM  405  C   VAL  45  34.747 39.381  37.173  1.00  26.01 AAAA
ATOM  406  O   VAL  45  34.901 40.603  37.199  1.00  25.56 AAAA
ATOM  407  N   PRO  46  33.566 38.820  37.457  1.00  26.96 AAAA
ATOM  408  CD  PRO  46  33.174 37.412  37.264  1.00  28.85 AAAA
ATOM  409  CA  PRO  46  32.446 39.616  37.959  1.00  26.67 AAAA
ATOM  410  CB  PRO  46  31.356 38.578  38.209  1.00  26.01 AAAA
ATOM  411  CG  PRO  46  31.679 37.474  37.282  1.00  24.45 AAAA
ATOM  412  C   PRO  46  32.822 40.338  39.234  1.00  26.63 AAAA
ATOM  413  O   PRO  46  33.536 39.788  40.054  1.00  29.14 AAAA
ATOM  414  N   ARG  47  32.427 41.600  39.348  1.00  28.89 AAAA
ATOM  415  H   ARG  47  31.947 41.987  38.584  1.00   0.00 AAAA
ATOM  416  CA  ARG  47  32.660 42.390  40.558  1.00  31.96 AAAA
ATOM  417  CB  ARG  47  33.439 43.660  40.232  1.00  38.74 AAAA
ATOM  418  CG  ARG  47  34.628 43.440  39.357  1.00  45.55 AAAA
ATOM  419  CD  ARG  47  35.882 43.853  40.069  1.00  53.22 AAAA
ATOM  420  NE  ARG  47  37.031 43.644  39.196  1.00  57.19 AAAA
ATOM  421  HE  ARG  47  36.970 42.863  38.611  1.00   0.00 AAAA
ATOM  422  CZ  ARG  47  38.126 44.395  39.204  1.00  59.13 AAAA
ATOM  423  NH1 ARG  47  39.033 44.235  38.246  1.00  58.34 AAAA
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ATOM	424	HH11	ARG	47	38.884	43.574	37.513	1.00	0.00 AAAA
ATOM	425	HH12	ARG	47	39.848	44.815	38.243	1.00	0.00 AAAA
ATOM	426	NH2	ARG	47	38.341	45.252	40.202	1.00	57.19 AAAA
ATOM	427	HH21	ARG	47	39.161	45.822	40.207	1.00	0.00 AAAA
ATOM	428	HH22	ARG	47	37.667	45.360	40.937	1.00	0.00 AAAA
ATOM	429	C	ARG	47	31.334	42.788	41.207	1.00	31.57 AAAA
ATOM	430	O	ARG	47	30.279	42.698	40.578	1.00	35.91 AAAA
ATOM	431	N	PRO	48	31.375	43.285	42.450	1.00	28.07 AAAA
ATOM	432	CD	PRO	48	30.207	43.903	43.087	1.00	29.27 AAAA
ATOM	433	CA	PRO	48	32.511	43.267	43.374	1.00	27.18 AAAA
ATOM	434	CB	PRO	48	31.948	43.916	44.635	1.00	25.16 AAAA
ATOM	435	CG	PRO	48	30.837	44.729	44.159	1.00	30.03 AAAA
ATOM	436	C	PRO	48	33.010	41.854	43.664	1.00	26.56 AAAA
ATOM	437	O	PRO	48	32.286	40.875	43.454	1.00	24.00 AAAA
ATOM	438	N	VAL	49	34.301	41.761	43.982	1.00	25.54 AAAA
ATOM	439	H	VAL	49	34.852	42.554	43.813	1.00	0.00 AAAA
ATOM	440	CA	VAL	49	34.911	40.547	44.520	1.00	21.73 AAAA
ATOM	441	CB	VAL	49	36.246	40.261	43.799	1.00	22.26 AAAA
ATOM	442	CG1	VAL	49	36.948	39.064	44.423	1.00	20.99 AAAA
ATOM	443	CG2	VAL	49	35.991	40.004	42.332	1.00	21.69 AAAA
ATOM	444	C	VAL	49	35.182	40.791	46.004	1.00	17.83 AAAA
ATOM	445	O	VAL	49	35.671	41.851	46.363	1.00	19.33 AAAA
ATOM	446	N	CYS	50	34.736	39.895	46.880	1.00	19.34 AAAA
ATOM	447	H	CYS	50	34.184	39.155	46.538	1.00	0.00 AAAA
ATOM	448	CA	CYS	50	35.030	40.082	48.305	1.00	19.43 AAAA
ATOM	449	CB	CYS	50	33.751	40.098	49.147	1.00	18.28 AAAA
ATOM	450	SG	CYS	50	32.923	38.524	49.247	1.00	22.22 AAAA
ATOM	451	C	CYS	50	36.016	39.068	48.892	1.00	18.64 AAAA
ATOM	452	O	CYS	50	36.363	39.141	50.067	1.00	21.41 AAAA
ATOM	453	N	ALA	51	36.527	38.174	48.064	1.00	16.97 AAAA
ATOM	454	H	ALA	51	36.204	38.132	47.146	1.00	0.00 AAAA
ATOM	455	CA	ALA	51	37.527	37.235	48.525	1.00	12.98 AAAA
ATOM	456	CB	ALA	51	36.899	36.197	49.434	1.00	15.92 AAAA
ATOM	457	C	ALA	51	38.156	36.559	47.345	1.00	17.22 AAAA
ATOM	458	O	ALA	51	37.489	36.264	46.361	1.00	18.29 AAAA
ATOM	459	N	VAL	52	39.454	36.304	47.447	1.00	16.94 AAAA
ATOM	460	H	VAL	52	39.935	36.706	48.201	1.00	0.00 AAAA
ATOM	461	CA	VAL	52	40.148	35.446	46.485	1.00	16.89 AAAA
ATOM	462	CB	VAL	52	41.325	36.226	45.769	1.00	14.71 AAAA
ATOM	463	CG1	VAL	52	42.104	35.295	44.864	1.00	14.13 AAAA
ATOM	464	CG2	VAL	52	40.784	37.417	44.983	1.00	15.31 AAAA
ATOM	465	C	VAL	52	40.711	34.245	47.249	1.00	15.15 AAAA
ATOM	466	O	VAL	52	41.310	34.403	48.326	1.00	18.19 AAAA

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ATOM  467  N   LEU  53  40.428 33.050  46.765  1.00  12.59 AAAA
ATOM  468  H   LEU  53  39.784 32.985  46.031  1.00   0.00 AAAA
ATOM  469  CA  LEU  53  41.081 31.873  47.287  1.00  14.74 AAAA
ATOM  470  CB  LEU  53  40.067 30.754  47.519  1.00  16.32 AAAA
ATOM  471  CG  LEU  53  39.487 30.583  48.937  1.00  18.05 AAAA
ATOM  472  CD1 LEU  53  38.986 31.897  49.449  1.00  16.19 AAAA
ATOM  473  CD2 LEU  53  38.370 29.542  48.921  1.00  15.85 AAAA
ATOM  474  C   LEU  53  42.186 31.408  46.327  1.00  20.78 AAAA
ATOM  475  O   LEU  53  41.992 31.395  45.109  1.00  15.78 AAAA
ATOM  476  N   LEU  54  43.389 31.205  46.873  1.00  19.44 AAAA
ATOM  477  H   LEU  54  43.473 31.356  47.838  1.00   0.00 AAAA
ATOM  478  CA  LEU  54  44.527 30.741  46.081  1.00  19.71 AAAA
ATOM  479  CB  LEU  54  45.728 31.698  46.209  1.00  19.22 AAAA
ATOM  480  CG  LEU  54  47.039 31.318  45.501  1.00  18.95 AAAA
ATOM  481  CD1 LEU  54  46.863 31.342  43.981  1.00  15.60 AAAA
ATOM  482  CD2 LEU  54  48.118 32.309  45.904  1.00  18.59 AAAA
ATOM  483  C   LEU  54  44.938 29.353  46.504  1.00  19.65 AAAA
ATOM  484  O   LEU  54  45.116 29.081  47.709  1.00  16.10 AAAA
ATOM  485  N   LEU  55  44.939 28.457  45.517  1.00  19.34 AAAA
ATOM  486  H   LEU  55  44.559 28.742  44.656  1.00   0.00 AAAA
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ATOM  488  CB  LEU  55  44.586 26.099  44.908  1.00  18.64 AAAA
ATOM  489  CG  LEU  55  44.979 24.625  45.027  1.00  20.11 AAAA
ATOM  490  CD1 LEU  55  44.716 24.084  46.435  1.00  22.00 AAAA
ATOM  491  CD2 LEU  55  44.183 23.827  44.019  1.00  22.10 AAAA
ATOM  492  C   LEU  55  46.882 27.044  45.105  1.00  22.01 AAAA
ATOM  493  O   LEU  55  47.107 27.348  43.930  1.00  19.66 AAAA
ATOM  494  N   PHE  56  47.836 26.685  45.959  1.00  21.06 AAAA
ATOM  495  H   PHE  56  47.572 26.417  46.861  1.00   0.00 AAAA
ATOM  496  CA  PHE  56  49.249 26.696  45.580  1.00  20.59 AAAA
ATOM  497  CB  PHE  56  49.883 28.048  45.948  1.00  16.88 AAAA
ATOM  498  CG  PHE  56  50.061 28.261  47.418  1.00  23.60 AAAA
ATOM  499  CD1 PHE  56  51.221 27.831  48.063  1.00  25.11 AAAA
ATOM  500  CD2 PHE  56  49.073 28.886  48.172  1.00  23.92 AAAA
ATOM  501  CE1 PHE  56  51.384 28.015  49.433  1.00  27.68 AAAA
ATOM  502  CE2 PHE  56  49.235 29.078  49.549  1.00  23.11 AAAA
ATOM  503  CZ  PHE  56  50.383 28.642  50.176  1.00  26.70 AAAA
ATOM  504  C   PHE  56  49.963 25.540  46.266  1.00  22.28 AAAA
ATOM  505  O   PHE  56  49.394 24.890  47.158  1.00  21.61 AAAA
ATOM  506  N   PRO  57  51.193 25.208  45.819  1.00  27.77 AAAA
ATOM  507  CD  PRO  57  51.930 25.733  44.651  1.00  25.94 AAAA
ATOM  508  CA  PRO  57  51.891 24.060  46.423  1.00  26.56 AAAA
ATOM  509  CB  PRO  57  52.538 23.377  45.216  1.00  27.54 AAAA
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ATOM	510	CG	PRO	57	52.668	24.507	44.160	1.00	29.50	AAAA
ATOM	511	C	PRO	57	52.906	24.439	47.523	1.00	23.83	AAAA
ATOM	512	O	PRO	57	53.565	25.481	47.460	1.00	23.72	AAAA
ATOM	513	N	ILE	58	52.874	23.689	48.615	1.00	27.04	AAAA
ATOM	514	H	ILE	58	52.184	23.055	48.691	1.00	0.00	AAAA
ATOM	515	CA	ILE	58	53.800	23.906	49.728	1.00	31.09	AAAA
ATOM	516	CB	ILE	58	53.241	23.338	51.064	1.00	32.61	AAAA
ATOM	517	CG2	ILE	58	54.204	23.642	52.226	1.00	31.31	AAAA
ATOM	518	CG1	ILE	58	51.875	23.949	51.363	1.00	30.84	AAAA
ATOM	519	CD	ILE	58	51.174	23.250	52.487	1.00	29.57	AAAA
ATOM	520	C	ILE	58	55.142	23.234	49.427	1.00	31.74	AAAA
ATOM	521	O	ILE	58	55.278	22.009	49.512	1.00	33.47	AAAA
ATOM	522	N	THR	59	56.072	24.022	48.910	1.00	33.63	AAAA
ATOM	523	H	THR	59	55.804	24.955	48.785	1.00	0.00	AAAA
ATOM	524	CA	THR	59	57.390	23.511	48.556	1.00	32.86	AAAA
ATOM	525	CB	THR	59	57.838	24.082	47.205	1.00	34.38	AAAA
ATOM	526	OG1	THR	59	57.864	25.513	47.284	1.00	30.05	AAAA
ATOM	527	HG1	THR	59	57.104	25.829	46.781	1.00	0.00	AAAA
ATOM	528	CG2	THR	59	56.877	23.658	46.091	1.00	34.44	AAAA
ATOM	529	C	THR	59	58.417	23.901	49.616	1.00	34.06	AAAA
ATOM	530	O	THR	59	58.157	24.786	50.429	1.00	30.95	AAAA
ATOM	531	N	GLU	60	59.593	23.271	49.578	1.00	36.00	AAAA
ATOM	532	H	GLU	60	59.653	22.488	48.995	1.00	0.00	AAAA
ATOM	533	CA	GLU	60	60.720	23.670	50.426	1.00	36.26	AAAA
ATOM	534	CB	GLU	60	61.980	22.903	50.033	1.00	42.18	AAAA
ATOM	535	CG	GLU	60	62.050	21.471	50.549	1.00	53.87	AAAA
ATOM	536	CD	GLU	60	63.296	20.736	50.068	1.00	59.16	AAAA
ATOM	537	OE1	GLU	60	64.348	20.813	50.747	1.00	63.06	AAAA
ATOM	538	OE2	GLU	60	63.225	20.091	48.996	1.00	63.28	AAAA
ATOM	539	C	GLU	60	60.982	25.153	50.256	1.00	35.56	AAAA
ATOM	540	O	GLU	60	61.101	25.890	51.228	1.00	37.03	AAAA
ATOM	541	N	LYS	61	60.986	25.582	48.999	1.00	35.28	AAAA
ATOM	542	H	LYS	61	60.883	24.902	48.307	1.00	0.00	AAAA
ATOM	543	CA	LYS	61	61.178	26.974	48.607	1.00	35.78	AAAA
ATOM	544	CB	LYS	61	61.079	27.060	47.088	1.00	38.62	AAAA
ATOM	545	CG	LYS	61	61.833	28.185	46.448	1.00	43.95	AAAA
ATOM	546	CD	LYS	61	62.080	27.843	44.990	1.00	46.20	AAAA
ATOM	547	CE	LYS	61	63.096	28.769	44.355	1.00	48.83	AAAA
ATOM	548	NZ	LYS	61	63.535	28.244	43.029	1.00	51.71	AAAA
ATOM	549	HZ1	LYS	61	62.705	28.054	42.432	1.00	0.00	AAAA
ATOM	550	HZ2	LYS	61	64.068	27.364	43.181	1.00	0.00	AAAA
ATOM	551	HZ3	LYS	61	64.154	28.942	42.568	1.00	0.00	AAAA
ATOM	552	C	LYS	61	60.115	27.877	49.246	1.00	36.67	AAAA

ATOM	553	O	LYS	61	60.425	28.913	49.836	1.00	36.93 AAAA
ATOM	554	N	TYR	62	58.859	27.447	49.168	1.00	35.28 AAAA
ATOM	555	H	TYR	62	58.677	26.650	48.629	1.00	0.00 AAAA
ATOM	556	CA	TYR	62	57.764	28.114	49.866	1.00	31.76 AAAA
ATOM	557	CB	TYR	62	56.460	27.342	49.626	1.00	31.61 AAAA
ATOM	558	CG	TYR	62	55.310	27.834	50.461	1.00	26.21 AAAA
ATOM	559	CD1	TYR	62	54.761	29.088	50.222	1.00	30.35 AAAA
ATOM	560	CE1	TYR	62	53.856	29.659	51.106	1.00	29.55 AAAA
ATOM	561	CD2	TYR	62	54.905	27.144	51.600	1.00	25.46 AAAA
ATOM	562	CE2	TYR	62	54.001	27.710	52.501	1.00	25.74 AAAA
ATOM	563	CZ	TYR	62	53.488	28.967	52.243	1.00	26.36 AAAA
ATOM	564	OH	TYR	62	52.641	29.589	53.116	1.00	26.54 AAAA
ATOM	565	HH	TYR	62	52.044	30.083	52.548	1.00	0.00 AAAA
ATOM	566	C	TYR	62	58.059	28.190	51.372	1.00	30.10 AAAA
ATOM	567	O	TYR	62	58.032	29.266	51.976	1.00	31.64 AAAA
ATOM	568	N	GLU	63	58.434	27.054	51.946	1.00	31.85 AAAA
ATOM	569	H	GLU	63	58.606	26.285	51.371	1.00	0.00 AAAA
ATOM	570	CA	GLU	63	58.638	26.941	53.387	1.00	34.36 AAAA
ATOM	571	CB	GLU	63	58.892	25.488	53.770	1.00	35.57 AAAA
ATOM	572	CG	GLU	63	57.659	24.624	53.771	1.00	36.98 AAAA
ATOM	573	CD	GLU	63	56.658	25.070	54.806	1.00	38.63 AAAA
ATOM	574	OE1	GLU	63	55.884	26.008	54.529	1.00	38.48 AAAA
ATOM	575	OE2	GLU	63	56.652	24.479	55.900	1.00	41.70 AAAA
ATOM	576	C	GLU	63	59.759	27.814	53.960	1.00	35.89 AAAA
ATOM	577	O	GLU	63	59.650	28.290	55.099	1.00	35.16 AAAA
ATOM	578	N	VAL	64	60.835	28.035	53.207	1.00	34.07 AAAA
ATOM	579	H	VAL	64	60.904	27.569	52.341	1.00	0.00 AAAA
ATOM	580	CA	VAL	64	61.879	28.901	53.744	1.00	32.48 AAAA
ATOM	581	CB	VAL	64	63.282	28.790	52.994	1.00	34.25 AAAA
ATOM	582	CG1	VAL	64	63.535	27.365	52.550	1.00	30.44 AAAA
ATOM	583	CG2	VAL	64	63.400	29.793	51.831	1.00	32.48 AAAA
ATOM	584	C	VAL	64	61.373	30.339	53.768	1.00	30.07 AAAA
ATOM	585	O	VAL	64	61.497	31.023	54.794	1.00	30.66 AAAA
ATOM	586	N	PHE	65	60.649	30.739	52.728	1.00	25.14 AAAA
ATOM	587	H	PHE	65	60.486	30.125	51.980	1.00	0.00 AAAA
ATOM	588	CA	PHE	65	60.092	32.077	52.726	1.00	27.03 AAAA
ATOM	589	CB	PHE	65	59.362	32.379	51.424	1.00	28.76 AAAA
ATOM	590	CG	PHE	65	58.726	33.738	51.403	1.00	30.67 AAAA
ATOM	591	CD1	PHE	65	59.449	34.847	50.985	1.00	31.43 AAAA
ATOM	592	CD2	PHE	65	57.462	33.930	51.945	1.00	31.83 AAAA
ATOM	593	CE1	PHE	65	58.933	36.127	51.122	1.00	33.66 AAAA
ATOM	594	CE2	PHE	65	56.940	35.203	52.090	1.00	31.50 AAAA
ATOM	595	CZ	PHE	65	57.677	36.306	51.681	1.00	34.79 AAAA

ATOM	596	C	PHE	65	59.127	32.255	53.898	1.00	27.51 AAAA
ATOM	597	O	PHE	65	59.178	33.253	54.602	1.00	28.94 AAAA
ATOM	598	N	ARG	66	58.233	31.294	54.087	1.00	29.57 AAAA
ATOM	599	H	ARG	66	58.211	30.557	53.439	1.00	0.00 AAAA
ATOM	600	CA	ARG	66	57.277	31.358	55.189	1.00	28.53 AAAA
ATOM	601	CB	ARG	66	56.409	30.103	55.210	1.00	28.27 AAAA
ATOM	602	CG	ARG	66	55.210	30.274	56.099	1.00	30.07 AAAA
ATOM	603	CD	ARG	66	54.586	28.967	56.414	1.00	34.44 AAAA
ATOM	604	NE	ARG	66	55.118	28.447	57.657	1.00	43.87 AAAA
ATOM	605	HE	ARG	66	55.420	29.082	58.336	1.00	0.00 AAAA
ATOM	606	CZ	ARG	66	55.251	27.159	57.915	1.00	47.87 AAAA
ATOM	607	NH1	ARG	66	55.743	26.760	59.077	1.00	56.13 AAAA
ATOM	608	HH11	ARG	66	55.911	27.432	59.798	1.00	0.00 AAAA
ATOM	609	HH12	ARG	66	55.849	25.785	59.271	1.00	0.00 AAAA
ATOM	610	NH2	ARG	66	54.831	26.267	57.034	1.00	54.07 AAAA
ATOM	611	HH21	ARG	66	54.902	25.291	57.249	1.00	0.00 AAAA
ATOM	612	HH22	ARG	66	54.365	26.557	56.200	1.00	0.00 AAAA
ATOM	613	C	ARG	66	57.923	31.562	56.574	1.00	28.57 AAAA
ATOM	614	O	ARG	66	57.495	32.431	57.349	1.00	26.47 AAAA
ATOM	615	N	THR	67	58.993	30.817	56.856	1.00	28.49 AAAA
ATOM	616	H	THR	67	59.242	30.128	56.207	1.00	0.00 AAAA
ATOM	617	CA	THR	67	59.711	30.970	58.127	1.00	26.81 AAAA
ATOM	618	CB	THR	67	60.732	29.895	58.333	1.00	27.83 AAAA
ATOM	619	OG1	THR	67	60.144	28.631	58.024	1.00	37.76 AAAA
ATOM	620	HG1	THR	67	60.091	28.441	57.077	1.00	0.00 AAAA
ATOM	621	CG2	THR	67	61.157	29.879	59.784	1.00	34.84 AAAA
ATOM	622	C	THR	67	60.425	32.297	58.270	1.00	24.31 AAAA
ATOM	623	O	THR	67	60.343	32.931	59.316	1.00	25.88 AAAA
ATOM	624	N	GLU	68	61.016	32.776	57.180	1.00	24.39 AAAA
ATOM	625	H	GLU	68	61.054	32.210	56.386	1.00	0.00 AAAA
ATOM	626	CA	GLU	68	61.576	34.117	57.165	1.00	26.47 AAAA
ATOM	627	CB	GLU	68	62.239	34.391	55.817	1.00	25.86 AAAA
ATOM	628	CG	GLU	68	63.442	33.483	55.597	1.00	34.86 AAAA
ATOM	629	CD	GLU	68	64.410	33.971	54.528	1.00	32.64 AAAA
ATOM	630	OE1	GLU	68	64.606	35.207	54.393	1.00	33.07 AAAA
ATOM	631	OE2	GLU	68	65.006	33.097	53.862	1.00	30.30 AAAA
ATOM	632	C	GLU	68	60.509	35.174	57.451	1.00	28.99 AAAA
ATOM	633	O	GLU	68	60.692	36.049	58.309	1.00	27.30 AAAA
ATOM	634	N	GLU	69	59.368	35.048	56.771	1.00	28.12 AAAA
ATOM	635	H	GLU	69	59.312	34.308	56.134	1.00	0.00 AAAA
ATOM	636	CA	GLU	69	58.241	35.966	56.952	1.00	24.58 AAAA
ATOM	637	CB	GLU	69	57.096	35.565	56.017	1.00	23.71 AAAA
ATOM	638	CG	GLU	69	55.847	36.368	56.196	1.00	22.11 AAAA

ATOM	639	CD	GLU	69	54.705	35.813	55.380	1.00	25.45 AAAA
ATOM	640	OE1	GLU	69	54.228	34.710	55.713	1.00	23.09 AAAA
ATOM	641	OE2	GLU	69	54.296	36.476	54.404	1.00	26.44 AAAA
ATOM	642	C	GLU	69	57.754	35.984	58.404	1.00	21.08 AAAA
ATOM	643	O	GLU	69	57.476	37.041	58.959	1.00	20.51 AAAA
ATOM	644	N	GLU	70	57.714	34.814	59.028	1.00	19.93 AAAA
ATOM	645	H	GLU	70	57.946	34.008	58.528	1.00	0.00 AAAA
ATOM	646	CA	GLU	70	57.309	34.721	60.419	1.00	24.42 AAAA
ATOM	647	CB	GLU	70	57.136	33.256	60.815	1.00	24.09 AAAA
ATOM	648	CG	GLU	70	56.382	33.079	62.114	1.00	25.29 AAAA
ATOM	649	CD	GLU	70	56.333	31.650	62.584	1.00	24.10 AAAA
ATOM	650	OE1	GLU	70	56.101	30.753	61.745	1.00	25.29 AAAA
ATOM	651	OE2	GLU	70	56.489	31.431	63.806	1.00	26.77 AAAA
ATOM	652	C	GLU	70	58.325	35.387	61.354	1.00	26.36 AAAA
ATOM	653	O	GLU	70	57.959	36.181	62.222	1.00	27.60 AAAA
ATOM	654	N	GLU	71	59.605	35.153	61.091	1.00	28.74 AAAA
ATOM	655	H	GLU	71	59.819	34.531	60.361	1.00	0.00 AAAA
ATOM	656	CA	GLU	71	60.684	35.764	61.873	1.00	31.06 AAAA
ATOM	657	CB	GLU	71	62.027	35.154	61.463	1.00	34.77 AAAA
ATOM	658	CG	GLU	71	62.138	33.657	61.762	1.00	45.41 AAAA
ATOM	659	CD	GLU	71	63.521	33.074	61.448	1.00	56.76 AAAA
ATOM	660	OE1	GLU	71	64.276	33.686	60.642	1.00	58.43 AAAA
ATOM	661	OE2	GLU	71	63.846	31.993	62.008	1.00	59.87 AAAA
ATOM	662	C	GLU	71	60.724	37.289	61.717	1.00	27.50 AAAA
ATOM	663	O	GLU	71	60.776	38.029	62.706	1.00	26.56 AAAA
ATOM	664	N	LYS	72	60.557	37.752	60.485	1.00	24.83 AAAA
ATOM	665	H	LYS	72	60.474	37.104	59.763	1.00	0.00 AAAA
ATOM	666	CA	LYS	72	60.517	39.184	60.216	1.00	27.68 AAAA
ATOM	667	CB	LYS	72	60.501	39.444	58.704	1.00	29.44 AAAA
ATOM	668	CG	LYS	72	60.634	40.917	58.346	1.00	35.91 AAAA
ATOM	669	CD	LYS	72	60.541	41.141	56.849	1.00	45.30 AAAA
ATOM	670	CE	LYS	72	59.768	42.427	56.528	1.00	50.61 AAAA
ATOM	671	NZ	LYS	72	58.357	42.379	57.040	1.00	52.85 AAAA
ATOM	672	HZ1	LYS	72	58.363	42.253	58.072	1.00	0.00 AAAA
ATOM	673	HZ2	LYS	72	57.867	41.574	56.600	1.00	0.00 AAAA
ATOM	674	HZ3	LYS	72	57.864	43.263	56.803	1.00	0.00 AAAA
ATOM	675	C	LYS	72	59.336	39.904	60.898	1.00	28.50 AAAA
ATOM	676	O	LYS	72	59.505	40.990	61.453	1.00	26.07 AAAA
ATOM	677	N	ILE	73	58.163	39.269	60.925	1.00	27.98 AAAA
ATOM	678	H	ILE	73	58.085	38.402	60.469	1.00	0.00 AAAA
ATOM	679	CA	ILE	73	57.014	39.870	61.586	1.00	30.16 AAAA
ATOM	680	CB	ILE	73	55.678	39.221	61.117	1.00	32.09 AAAA
ATOM	681	CG2	ILE	73	54.518	39.615	62.075	1.00	28.61 AAAA

ATOM	682	CG1	ILE	73	55.362	39.681	59.683	1.00	30.18 AAAA
ATOM	683	CD	ILE	73	54.303	38.839	58.964	1.00	28.53 AAAA
ATOM	684	C	ILE	73	57.117	39.813	63.117	1.00	32.56 AAAA
ATOM	685	O	ILE	73	56.658	40.716	63.817	1.00	31.86 AAAA
ATOM	686	N	LYS	74	57.758	38.778	63.637	1.00	34.68 AAAA
ATOM	687	H	LYS	74	58.046	38.050	63.047	1.00	0.00 AAAA
ATOM	688	CA	LYS	74	57.996	38.703	65.070	1.00	38.56 AAAA
ATOM	689	CB	LYS	74	58.455	37.292	65.439	1.00	41.67 AAAA
ATOM	690	CG	LYS	74	57.374	36.221	65.241	1.00	44.95 AAAA
ATOM	691	CD	LYS	74	57.997	34.832	65.096	1.00	49.48 AAAA
ATOM	692	CE	LYS	74	57.330	33.811	66.003	1.00	50.67 AAAA
ATOM	693	NZ	LYS	74	55.898	33.585	65.661	1.00	53.91 AAAA
ATOM	694	HZ1	LYS	74	55.817	32.986	64.817	1.00	0.00 AAAA
ATOM	695	HZ2	LYS	74	55.437	34.499	65.477	1.00	0.00 AAAA
ATOM	696	HZ3	LYS	74	55.431	33.117	66.465	1.00	0.00 AAAA
ATOM	697	C	LYS	74	59.014	39.764	65.542	1.00	40.96 AAAA
ATOM	698	O	LYS	74	58.880	40.327	66.627	1.00	41.57 AAAA
ATOM	699	N	SER	75	59.955	40.122	64.675	1.00	41.35 AAAA
ATOM	700	H	SER	75	60.035	39.595	63.850	1.00	0.00 AAAA
ATOM	701	CA	SER	75	60.878	41.219	64.969	1.00	43.19 AAAA
ATOM	702	CB	SER	75	62.102	41.163	64.046	1.00	44.46 AAAA
ATOM	703	OG	SER	75	62.467	42.453	63.571	1.00	46.60 AAAA
ATOM	704	HG	SER	75	62.724	43.054	64.265	1.00	0.00 AAAA
ATOM	705	C	SER	75	60.217	42.587	64.828	1.00	44.73 AAAA
ATOM	706	O	SER	75	60.382	43.463	65.685	1.00	48.52 AAAA
ATOM	707	N	GLN	76	59.518	42.785	63.715	1.00	41.15 AAAA
ATOM	708	H	GLN	76	59.362	42.019	63.126	1.00	0.00 AAAA
ATOM	709	CA	GLN	76	59.108	44.117	63.303	1.00	38.47 AAAA
ATOM	710	CB	GLN	76	59.590	44.350	61.875	1.00	41.98 AAAA
ATOM	711	CG	GLN	76	58.516	44.515	60.824	1.00	43.50 AAAA
ATOM	712	CD	GLN	76	59.007	45.325	59.660	1.00	44.41 AAAA
ATOM	713	OE1	GLN	76	60.119	45.841	59.675	1.00	43.52 AAAA
ATOM	714	NE2	GLN	76	58.182	45.449	58.644	1.00	48.56 AAAA
ATOM	715	HE21	GLN	76	57.294	45.040	58.703	1.00	0.00 AAAA
ATOM	716	HE22	GLN	76	58.569	45.963	57.911	1.00	0.00 AAAA
ATOM	717	C	GLN	76	57.606	44.411	63.444	1.00	35.54 AAAA
ATOM	718	O	GLN	76	57.170	45.564	63.305	1.00	33.94 AAAA
ATOM	719	N	GLY	77	56.852	43.400	63.867	1.00	31.89 AAAA
ATOM	720	H	GLY	77	57.272	42.529	63.999	1.00	0.00 AAAA
ATOM	721	CA	GLY	77	55.429	43.573	64.095	1.00	29.13 AAAA
ATOM	722	C	GLY	77	54.617	43.816	62.833	1.00	26.74 AAAA
ATOM	723	O	GLY	77	55.167	44.016	61.743	1.00	25.60 AAAA
ATOM	724	N	GLN	78	53.297	43.804	62.985	1.00	24.43 AAAA

ATOM	725	H	GLN	78	52.921	43.551	63.845	1.00	0.00 AAAA
ATOM	726	CA	GLN	78	52.385	44.167	61.900	1.00	22.99 AAAA
ATOM	727	CB	GLN	78	52.217	42.990	60.942	1.00	23.16 AAAA
ATOM	728	CG	GLN	78	51.534	41.796	61.602	1.00	23.53 AAAA
ATOM	729	CD	GLN	78	51.099	40.730	60.627	1.00	17.76 AAAA
ATOM	730	OE1	GLN	78	51.185	40.900	59.408	1.00	16.41 AAAA
ATOM	731	NE2	GLN	78	50.685	39.597	61.160	1.00	15.76 AAAA
ATOM	732	HE21	GLN	78	50.651	39.510	62.138	1.00	0.00 AAAA
ATOM	733	HE22	GLN	78	50.447	38.904	60.516	1.00	0.00 AAAA
ATOM	734	C	GLN	78	51.032	44.498	62.540	1.00	25.68 AAAA
ATOM	735	O	GLN	78	50.757	44.098	63.676	1.00	26.20 AAAA
ATOM	736	N	ASP	79	50.185	45.223	61.826	1.00	23.31 AAAA
ATOM	737	H	ASP	79	50.483	45.471	60.932	1.00	0.00 AAAA
ATOM	738	CA	ASP	79	48.838	45.466	62.326	1.00	25.37 AAAA
ATOM	739	CB	ASP	79	48.386	46.880	61.962	1.00	24.45 AAAA
ATOM	740	CG	ASP	79	49.211	47.959	62.672	1.00	28.21 AAAA
ATOM	741	OD1	ASP	79	50.071	47.598	63.516	1.00	26.57 AAAA
ATOM	742	OD2	ASP	79	49.018	49.158	62.360	1.00	28.96 AAAA
ATOM	743	C	ASP	79	47.824	44.428	61.824	1.00	27.86 AAAA
ATOM	744	O	ASP	79	47.821	44.089	60.638	1.00	26.79 AAAA
ATOM	745	N	VAL	80	47.133	43.784	62.770	1.00	27.61 AAAA
ATOM	746	H	VAL	80	47.439	43.860	63.690	1.00	0.00 AAAA
ATOM	747	CA	VAL	80	45.971	42.948	62.457	1.00	25.15 AAAA
ATOM	748	CB	VAL	80	46.207	41.477	62.840	1.00	23.30 AAAA
ATOM	749	CG1	VAL	80	45.007	40.651	62.450	1.00	25.36 AAAA
ATOM	750	CG2	VAL	80	47.438	40.941	62.141	1.00	22.16 AAAA
ATOM	751	C	VAL	80	44.739	43.452	63.201	1.00	24.57 AAAA
ATOM	752	O	VAL	80	44.644	43.295	64.413	1.00	22.28 AAAA
ATOM	753	N	THR	81	43.813	44.073	62.474	1.00	24.26 AAAA
ATOM	754	H	THR	81	44.032	44.168	61.520	1.00	0.00 AAAA
ATOM	755	CA	THR	81	42.571	44.571	63.078	1.00	27.38 AAAA
ATOM	756	CB	THR	81	41.646	45.222	62.002	1.00	28.45 AAAA
ATOM	757	OG1	THR	81	40.486	45.773	62.625	1.00	39.19 AAAA
ATOM	758	HG1	THR	81	40.319	46.609	62.180	1.00	0.00 AAAA
ATOM	759	CG2	THR	81	41.194	44.214	60.995	1.00	31.37 AAAA
ATOM	760	C	THR	81	41.806	43.489	63.857	1.00	27.08 AAAA
ATOM	761	O	THR	81	41.840	42.302	63.503	1.00	25.09 AAAA
ATOM	762	N	SER	82	41.203	43.873	64.978	1.00	26.66 AAAA
ATOM	763	H	SER	82	41.296	44.807	65.280	1.00	0.00 AAAA
ATOM	764	CA	SER	82	40.425	42.912	65.764	1.00	27.37 AAAA
ATOM	765	CB	SER	82	39.992	43.506	67.113	1.00	27.54 AAAA
ATOM	766	OG	SER	82	39.073	44.565	66.929	1.00	32.64 AAAA
ATOM	767	HG	SER	82	38.160	44.228	66.813	1.00	0.00 AAAA

ATOM	768	C	SER	82	39.192	42.404	65.014	1.00	23.10 AAAA
ATOM	769	O	SER	82	38.628	41.385	65.374	1.00	24.03 AAAA
ATOM	770	N	SER	83	38.790	43.105	63.962	1.00	25.20 AAAA
ATOM	771	H	SER	83	39.201	43.984	63.811	1.00	0.00 AAAA
ATOM	772	CA	SER	83	37.682	42.640	63.126	1.00	27.82 AAAA
ATOM	773	CB	SER	83	37.339	43.671	62.046	1.00	31.00 AAAA
ATOM	774	OG	SER	83	37.002	44.929	62.602	1.00	44.02 AAAA
ATOM	775	HG	SER	83	37.201	45.629	61.989	1.00	0.00 AAAA
ATOM	776	C	SER	83	38.014	41.315	62.432	1.00	27.88 AAAA
ATOM	777	O	SER	83	37.114	40.593	61.985	1.00	29.20 AAAA
ATOM	778	N	VAL	84	39.303	41.087	62.191	1.00	23.83 AAAA
ATOM	779	H	VAL	84	39.969	41.681	62.581	1.00	0.00 AAAA
ATOM	780	CA	VAL	84	39.723	39.940	61.402	1.00	20.73 AAAA
ATOM	781	CB	VAL	84	41.231	39.971	61.120	1.00	20.85 AAAA
ATOM	782	CG1	VAL	84	41.681	38.634	60.533	1.00	21.87 AAAA
ATOM	783	CG2	VAL	84	41.536	41.072	60.152	1.00	20.91 AAAA
ATOM	784	C	VAL	84	39.383	38.619	62.064	1.00	19.51 AAAA
ATOM	785	O	VAL	84	39.745	38.379	63.206	1.00	20.68 AAAA
ATOM	786	N	TYR	85	38.640	37.780	61.352	1.00	19.17 AAAA
ATOM	787	H	TYR	85	38.292	38.118	60.502	1.00	0.00 AAAA
ATOM	788	CA	TYR	85	38.314	36.450	61.837	1.00	18.54 AAAA
ATOM	789	CB	TYR	85	36.921	36.008	61.331	1.00	18.00 AAAA
ATOM	790	CG	TYR	85	36.466	34.641	61.825	1.00	15.07 AAAA
ATOM	791	CD1	TYR	85	35.489	34.517	62.828	1.00	18.67 AAAA
ATOM	792	CE1	TYR	85	35.074	33.258	63.284	1.00	13.74 AAAA
ATOM	793	CD2	TYR	85	37.012	33.469	61.299	1.00	14.59 AAAA
ATOM	794	CE2	TYR	85	36.619	32.219	61.754	1.00	14.12 AAAA
ATOM	795	CZ	TYR	85	35.637	32.122	62.741	1.00	15.13 AAAA
ATOM	796	OH	TYR	85	35.201	30.885	63.110	1.00	15.74 AAAA
ATOM	797	HH	TYR	85	34.434	30.990	63.679	1.00	0.00 AAAA
ATOM	798	C	TYR	85	39.388	35.494	61.339	1.00	18.88 AAAA
ATOM	799	O	TYR	85	39.503	35.234	60.137	1.00	17.70 AAAA
ATOM	800	N	PHE	86	40.173	34.976	62.269	1.00	17.16 AAAA
ATOM	801	H	PHE	86	40.037	35.264	63.201	1.00	0.00 AAAA
ATOM	802	CA	PHE	86	41.227	34.031	61.938	1.00	17.14 AAAA
ATOM	803	CB	PHE	86	42.609	34.633	62.294	1.00	18.81 AAAA
ATOM	804	CG	PHE	86	43.792	33.771	61.884	1.00	17.09 AAAA
ATOM	805	CD1	PHE	86	43.981	33.389	60.551	1.00	12.60 AAAA
ATOM	806	CD2	PHE	86	44.690	33.306	62.854	1.00	16.07 AAAA
ATOM	807	CE1	PHE	86	45.016	32.547	60.198	1.00	14.83 AAAA
ATOM	808	CE2	PHE	86	45.725	32.467	62.520	1.00	14.81 AAAA
ATOM	809	CZ	PHE	86	45.892	32.070	61.188	1.00	17.97 AAAA
ATOM	810	C	PHE	86	41.002	32.701	62.650	1.00	15.56 AAAA

ATOM	811	O	PHE	86	40.664	32.654	63.837	1.00	17.84 AAAA
ATOM	812	N	MET	87	41.296	31.626	61.931	1.00	13.32 AAAA
ATOM	813	H	MET	87	41.818	31.793	61.129	1.00	0.00 AAAA
ATOM	814	CA	MET	87	41.169	30.274	62.428	1.00	14.74 AAAA
ATOM	815	CB	MET	87	39.993	29.606	61.716	1.00	16.52 AAAA
ATOM	816	CG	MET	87	39.985	28.110	61.685	1.00	22.12 AAAA
ATOM	817	SD	MET	87	38.551	27.590	60.704	1.00	27.94 AAAA
ATOM	818	CE	MET	87	37.291	27.521	61.947	1.00	28.36 AAAA
ATOM	819	C	MET	87	42.462	29.523	62.118	1.00	18.15 AAAA
ATOM	820	O	MET	87	42.961	29.560	60.983	1.00	16.50 AAAA
ATOM	821	N	LYS	88	42.988	28.824	63.115	1.00	17.57 AAAA
ATOM	822	H	LYS	88	42.584	28.893	64.004	1.00	0.00 AAAA
ATOM	823	CA	LYS	88	44.121	27.929	62.896	1.00	18.62 AAAA
ATOM	824	CB	LYS	88	44.778	27.577	64.237	1.00	19.52 AAAA
ATOM	825	CG	LYS	88	45.534	28.739	64.872	1.00	24.91 AAAA
ATOM	826	CD	LYS	88	46.825	29.043	64.112	1.00	24.92 AAAA
ATOM	827	CE	LYS	88	47.815	27.882	64.223	1.00	24.92 AAAA
ATOM	828	NZ	LYS	88	48.821	27.887	63.118	1.00	25.56 AAAA
ATOM	829	HZ1	LYS	88	49.214	28.843	63.013	1.00	0.00 AAAA
ATOM	830	HZ2	LYS	88	48.349	27.612	62.237	1.00	0.00 AAAA
ATOM	831	HZ3	LYS	88	49.584	27.213	63.330	1.00	0.00 AAAA
ATOM	832	C	LYS	88	43.749	26.644	62.146	1.00	19.29 AAAA
ATOM	833	O	LYS	88	42.616	26.158	62.220	1.00	21.43 AAAA
ATOM	834	N	GLN	89	44.728	26.082	61.450	1.00	16.58 AAAA
ATOM	835	H	GLN	89	45.585	26.556	61.420	1.00	0.00 AAAA
ATOM	836	CA	GLN	89	44.599	24.779	60.819	1.00	17.18 AAAA
ATOM	837	CB	GLN	89	45.271	24.813	59.456	1.00	18.38 AAAA
ATOM	838	CG	GLN	89	45.350	23.485	58.760	1.00	18.24 AAAA
ATOM	839	CD	GLN	89	45.853	23.651	57.355	1.00	24.51 AAAA
ATOM	840	OE1	GLN	89	45.367	24.502	56.594	1.00	26.71 AAAA
ATOM	841	NE2	GLN	89	46.881	22.904	57.017	1.00	25.25 AAAA
ATOM	842	HE21	GLN	89	47.154	22.895	56.079	1.00	0.00 AAAA
ATOM	843	HE22	GLN	89	47.349	22.434	57.721	1.00	0.00 AAAA
ATOM	844	C	GLN	89	45.241	23.691	61.658	1.00	20.11 AAAA
ATOM	845	O	GLN	89	46.413	23.781	61.998	1.00	22.59 AAAA
ATOM	846	N	THR	90	44.517	22.603	61.869	1.00	18.50 AAAA
ATOM	847	H	THR	90	43.585	22.629	61.598	1.00	0.00 AAAA
ATOM	848	CA	THR	90	45.072	21.439	62.550	1.00	21.73 AAAA
ATOM	849	CB	THR	90	44.283	21.115	63.812	1.00	24.69 AAAA
ATOM	850	OG1	THR	90	42.924	20.794	63.464	1.00	30.17 AAAA
ATOM	851	HG1	THR	90	42.428	21.483	62.994	1.00	0.00 AAAA
ATOM	852	CG2	THR	90	44.301	22.312	64.752	1.00	28.14 AAAA
ATOM	853	C	THR	90	45.102	20.191	61.676	1.00	24.47 AAAA

ATOM	854	O	THR	90	45.846	19.252	61.956	1.00	28.80 AAAA
ATOM	855	N	ILE	91	44.271	20.171	60.632	1.00	22.87 AAAA
ATOM	856	H	ILE	91	43.593	20.871	60.590	1.00	0.00 AAAA
ATOM	857	CA	ILE	91	44.258	19.096	59.633	1.00	22.19 AAAA
ATOM	858	CB	ILE	91	42.921	18.326	59.639	1.00	22.46 AAAA
ATOM	859	CG2	ILE	91	42.989	17.157	58.695	1.00	18.95 AAAA
ATOM	860	CG1	ILE	91	42.591	17.837	61.050	1.00	24.03 AAAA
ATOM	861	CD	ILE	91	41.259	17.099	61.123	1.00	24.83 AAAA
ATOM	862	C	ILE	91	44.422	19.717	58.245	1.00	23.19 AAAA
ATOM	863	O	ILE	91	43.626	20.562	57.846	1.00	23.87 AAAA
ATOM	864	N	SER	92	45.413	19.266	57.487	1.00	23.54 AAAA
ATOM	865	H	SER	92	45.791	18.403	57.734	1.00	0.00 AAAA
ATOM	866	CA	SER	92	45.793	19.943	56.248	1.00	23.64 AAAA
ATOM	867	CB	SER	92	47.119	19.387	55.737	1.00	25.35 AAAA
ATOM	868	OG	SER	92	46.993	18.012	55.412	1.00	35.43 AAAA
ATOM	869	HG	SER	92	46.797	17.917	54.451	1.00	0.00 AAAA
ATOM	870	C	SER	92	44.735	19.799	55.165	1.00	22.11 AAAA
ATOM	871	O	SER	92	44.459	20.730	54.423	1.00	22.66 AAAA
ATOM	872	N	ASN	93	44.099	18.644	55.117	1.00	21.74 AAAA
ATOM	873	H	ASN	93	44.414	17.915	55.705	1.00	0.00 AAAA
ATOM	874	CA	ASN	93	43.007	18.434	54.183	1.00	27.91 AAAA
ATOM	875	CB	ASN	93	42.571	16.979	54.207	1.00	35.85 AAAA
ATOM	876	CG	ASN	93	43.673	16.057	53.764	1.00	47.97 AAAA
ATOM	877	OD1	ASN	93	44.253	16.233	52.684	1.00	52.33 AAAA
ATOM	878	ND2	ASN	93	44.076	15.160	54.652	1.00	54.60 AAAA
ATOM	879	HD21	ASN	93	43.681	15.126	55.548	1.00	0.00 AAAA
ATOM	880	HD22	ASN	93	44.780	14.587	54.290	1.00	0.00 AAAA
ATOM	881	C	ASN	93	41.793	19.323	54.428	1.00	25.96 AAAA
ATOM	882	O	ASN	93	40.897	19.373	53.585	1.00	24.44 AAAA
ATOM	883	N	ALA	94	41.760	19.994	55.583	1.00	24.06 AAAA
ATOM	884	H	ALA	94	42.454	19.832	56.247	1.00	0.00 AAAA
ATOM	885	CA	ALA	94	40.690	20.934	55.921	1.00	21.31 AAAA
ATOM	886	CB	ALA	94	40.516	21.022	57.440	1.00	16.74 AAAA
ATOM	887	C	ALA	94	40.940	22.325	55.354	1.00	19.83 AAAA
ATOM	888	O	ALA	94	40.089	23.195	55.464	1.00	18.59 AAAA
ATOM	889	N	CYS	95	42.103	22.544	54.744	1.00	17.72 AAAA
ATOM	890	H	CYS	95	42.699	21.784	54.585	1.00	0.00 AAAA
ATOM	891	CA	CYS	95	42.480	23.899	54.322	1.00	17.66 AAAA
ATOM	892	CB	CYS	95	43.908	23.905	53.752	1.00	17.95 AAAA
ATOM	893	SG	CYS	95	44.087	23.036	52.185	1.00	18.91 AAAA
ATOM	894	C	CYS	95	41.503	24.581	53.325	1.00	14.55 AAAA
ATOM	895	O	CYS	95	41.304	25.792	53.388	1.00	12.57 AAAA
ATOM	896	N	GLY	96	40.878	23.808	52.439	1.00	16.10 AAAA

ATOM	897	H	GLY	96	41.029	22.839	52.444	1.00	0.00 AAAA
ATOM	898	CA	GLY	96	39.900	24.384	51.525	1.00	14.24 AAAA
ATOM	899	C	GLY	96	38.660	24.858	52.274	1.00	13.81 AAAA
ATOM	900	O	GLY	96	38.220	25.996	52.109	1.00	12.17 AAAA
ATOM	901	N	THR	97	38.141	24.009	53.164	1.00	13.86 AAAA
ATOM	902	H	THR	97	38.518	23.101	53.219	1.00	0.00 AAAA
ATOM	903	CA	THR	97	37.033	24.406	54.037	1.00	14.26 AAAA
ATOM	904	CB	THR	97	36.597	23.257	54.922	1.00	14.44 AAAA
ATOM	905	OG1	THR	97	36.020	22.234	54.099	1.00	17.53 AAAA
ATOM	906	HG1	THR	97	35.307	21.820	54.623	1.00	0.00 AAAA
ATOM	907	CG2	THR	97	35.580	23.736	55.981	1.00	16.30 AAAA
ATOM	908	C	THR	97	37.383	25.596	54.927	1.00	14.53 AAAA
ATOM	909	O	THR	97	36.600	26.539	55.051	1.00	13.21 AAAA
ATOM	910	N	ILE	98	38.603	25.614	55.455	1.00	14.07 AAAA
ATOM	911	H	ILE	98	39.176	24.833	55.314	1.00	0.00 AAAA
ATOM	912	CA	ILE	98	39.058	26.755	56.242	1.00	13.22 AAAA
ATOM	913	CB	ILE	98	40.417	26.468	56.920	1.00	12.18 AAAA
ATOM	914	CG2	ILE	98	40.910	27.704	57.610	1.00	11.29 AAAA
ATOM	915	CG1	ILE	98	40.260	25.356	57.946	1.00	12.23 AAAA
ATOM	916	CD	ILE	98	41.556	24.629	58.268	1.00	15.43 AAAA
ATOM	917	C	ILE	98	39.156	28.017	55.404	1.00	11.49 AAAA
ATOM	918	O	ILE	98	38.755	29.093	55.853	1.00	12.87 AAAA
ATOM	919	N	GLY	99	39.597	27.885	54.157	1.00	11.22 AAAA
ATOM	920	H	GLY	99	39.875	27.007	53.832	1.00	0.00 AAAA
ATOM	921	CA	GLY	99	39.628	29.044	53.281	1.00	9.85 AAAA
ATOM	922	C	GLY	99	38.244	29.632	53.007	1.00	9.08 AAAA
ATOM	923	O	GLY	99	38.036	30.835	53.040	1.00	9.42 AAAA
ATOM	924	N	LEU	100	37.277	28.747	52.841	1.00	12.12 AAAA
ATOM	925	H	LEU	100	37.522	27.794	52.840	1.00	0.00 AAAA
ATOM	926	CA	LEU	100	35.900	29.148	52.602	1.00	15.16 AAAA
ATOM	927	CB	LEU	100	35.078	27.891	52.267	1.00	18.24 AAAA
ATOM	928	CG	LEU	100	33.723	28.022	51.575	1.00	24.26 AAAA
ATOM	929	CD1	LEU	100	33.831	29.058	50.465	1.00	26.09 AAAA
ATOM	930	CD2	LEU	100	33.289	26.660	51.015	1.00	21.41 AAAA
ATOM	931	C	LEU	100	35.347	29.870	53.838	1.00	13.53 AAAA
ATOM	932	O	LEU	100	34.837	30.980	53.740	1.00	14.20 AAAA
ATOM	933	N	ILE	101	35.598	29.300	55.013	1.00	13.77 AAAA
ATOM	934	H	ILE	101	36.051	28.431	55.000	1.00	0.00 AAAA
ATOM	935	CA	ILE	101	35.205	29.911	56.281	1.00	12.23 AAAA
ATOM	936	CB	ILE	101	35.571	28.996	57.462	1.00	13.26 AAAA
ATOM	937	CG2	ILE	101	35.402	29.734	58.786	1.00	15.98 AAAA
ATOM	938	CG1	ILE	101	34.697	27.732	57.405	1.00	11.45 AAAA
ATOM	939	CD	ILE	101	34.958	26.730	58.500	1.00	16.30 AAAA

ATOM	940	C	ILE	101	35.829	31.283	56.483	1.00	14.03 AAAA
ATOM	941	O	ILE	101	35.126	32.245	56.782	1.00	12.42 AAAA
ATOM	942	N	HIS	102	37.120	31.421	56.177	1.00	13.04 AAAA
ATOM	943	H	HIS	102	37.593	30.615	55.893	1.00	0.00 AAAA
ATOM	944	CA	HIS	102	37.769	32.738	56.239	1.00	10.47 AAAA
ATOM	945	CB	HIS	102	39.265	32.613	55.897	1.00	13.05 AAAA
ATOM	946	CG	HIS	102	40.107	32.125	57.030	1.00	10.70 AAAA
ATOM	947	CD2	HIS	102	40.153	32.499	58.332	1.00	13.91 AAAA
ATOM	948	ND1	HIS	102	41.088	31.168	56.877	1.00	11.81 AAAA
ATOM	949	HD1	HIS	102	41.295	30.682	56.041	1.00	0.00 AAAA
ATOM	950	CE1	HIS	102	41.708	30.974	58.028	1.00	11.88 AAAA
ATOM	951	NE2	HIS	102	41.162	31.778	58.927	1.00	15.13 AAAA
ATOM	952	HE2	HIS	102	41.468	31.860	59.843	1.00	0.00 AAAA
ATOM	953	C	HIS	102	37.125	33.757	55.291	1.00	11.19 AAAA
ATOM	954	O	HIS	102	36.969	34.935	55.640	1.00	12.51 AAAA
ATOM	955	N	ALA	103	36.844	33.328	54.055	1.00	13.10 AAAA
ATOM	956	H	ALA	103	37.020	32.394	53.814	1.00	0.00 AAAA
ATOM	957	CA	ALA	103	36.264	34.227	53.056	1.00	10.96 AAAA
ATOM	958	CB	ALA	103	36.140	33.504	51.703	1.00	9.09 AAAA
ATOM	959	C	ALA	103	34.881	34.712	53.531	1.00	15.64 AAAA
ATOM	960	O	ALA	103	34.558	35.899	53.440	1.00	18.87 AAAA
ATOM	961	N	ILE	104	34.073	33.787	54.047	1.00	15.87 AAAA
ATOM	962	H	ILE	104	34.402	32.864	54.115	1.00	0.00 AAAA
ATOM	963	CA	ILE	104	32.701	34.118	54.460	1.00	15.42 AAAA
ATOM	964	CB	ILE	104	31.813	32.829	54.537	1.00	13.84 AAAA
ATOM	965	CG2	ILE	104	30.365	33.200	54.915	1.00	18.30 AAAA
ATOM	966	CG1	ILE	104	31.822	32.081	53.204	1.00	13.16 AAAA
ATOM	967	CD	ILE	104	31.510	32.966	51.997	1.00	14.57 AAAA
ATOM	968	C	ILE	104	32.677	34.862	55.822	1.00	15.78 AAAA
ATOM	969	O	ILE	104	32.051	35.922	55.942	1.00	16.92 AAAA
ATOM	970	N	ALA	105	33.411	34.345	56.813	1.00	16.50 AAAA
ATOM	971	H	ALA	105	33.832	33.486	56.631	1.00	0.00 AAAA
ATOM	972	CA	ALA	105	33.529	34.998	58.121	1.00	17.00 AAAA
ATOM	973	CB	ALA	105	34.492	34.239	59.022	1.00	16.84 AAAA
ATOM	974	C	ALA	105	33.963	36.448	58.039	1.00	20.96 AAAA
ATOM	975	O	ALA	105	33.452	37.298	58.776	1.00	23.63 AAAA
ATOM	976	N	ASN	106	34.869	36.760	57.117	1.00	16.81 AAAA
ATOM	977	H	ASN	106	35.225	36.046	56.549	1.00	0.00 AAAA
ATOM	978	CA	ASN	106	35.347	38.132	57.002	1.00	17.98 AAAA
ATOM	979	CB	ASN	106	36.825	38.128	56.611	1.00	17.74 AAAA
ATOM	980	CG	ASN	106	37.707	37.690	57.755	1.00	14.08 AAAA
ATOM	981	OD1	ASN	106	37.977	38.463	58.655	1.00	17.07 AAAA
ATOM	982	ND2	ASN	106	38.042	36.415	57.795	1.00	14.28 AAAA

ATOM	983	HD21	ASN	106	37.713	35.831	57.096	1.00	0.00	AAAA
ATOM	984	HD22	ASN	106	38.611	36.181	58.571	1.00	0.00	AAAA
ATOM	985	C	ASN	106	34.522	39.008	56.048	1.00	19.28	AAAA
ATOM	986	O	ASN	106	34.898	40.129	55.748	1.00	17.22	AAAA
ATOM	987	N	ASN	107	33.389	38.478	55.584	1.00	21.43	AAAA
ATOM	988	H	ASN	107	33.219	37.539	55.782	1.00	0.00	AAAA
ATOM	989	CA	ASN	107	32.445	39.213	54.739	1.00	21.54	AAAA
ATOM	990	CB	ASN	107	32.611	38.786	53.296	1.00	18.81	AAAA
ATOM	991	CG	ASN	107	33.897	39.275	52.710	1.00	21.63	AAAA
ATOM	992	OD1	ASN	107	34.079	40.472	52.495	1.00	22.45	AAAA
ATOM	993	ND2	ASN	107	34.833	38.368	52.514	1.00	17.35	AAAA
ATOM	994	HD21	ASN	107	34.605	37.441	52.622	1.00	0.00	AAAA
ATOM	995	HD22	ASN	107	35.709	38.734	52.238	1.00	0.00	AAAA
ATOM	996	C	ASN	107	31.006	38.927	55.177	1.00	25.95	AAAA
ATOM	997	O	ASN	107	30.073	39.091	54.399	1.00	26.08	AAAA
ATOM	998	N	LYS	108	30.856	38.514	56.435	1.00	27.04	AAAA
ATOM	999	H	LYS	108	31.660	38.510	56.994	1.00	0.00	AAAA
ATOM	1000	CA	LYS	108	29.611	37.983	56.984	1.00	30.55	AAAA
ATOM	1001	CB	LYS	108	29.821	37.766	58.483	1.00	31.98	AAAA
ATOM	1002	CG	LYS	108	28.880	36.783	59.136	1.00	39.13	AAAA
ATOM	1003	CD	LYS	108	29.420	36.324	60.493	1.00	44.06	AAAA
ATOM	1004	CE	LYS	108	29.708	37.491	61.442	1.00	45.91	AAAA
ATOM	1005	NZ	LYS	108	29.833	37.023	62.849	1.00	46.37	AAAA
ATOM	1006	HZ1	LYS	108	30.640	36.383	62.912	1.00	0.00	AAAA
ATOM	1007	HZ2	LYS	108	28.963	36.525	63.134	1.00	0.00	AAAA
ATOM	1008	HZ3	LYS	108	29.992	37.838	63.478	1.00	0.00	AAAA
ATOM	1009	C	LYS	108	28.400	38.907	56.732	1.00	30.21	AAAA
ATOM	1010	O	LYS	108	27.330	38.462	56.309	1.00	28.80	AAAA
ATOM	1011	N	ASP	109	28.647	40.206	56.792	1.00	31.65	AAAA
ATOM	1012	H	ASP	109	29.573	40.464	56.950	1.00	0.00	AAAA
ATOM	1013	CA	ASP	109	27.601	41.191	56.575	1.00	34.09	AAAA
ATOM	1014	CB	ASP	109	27.982	42.501	57.274	1.00	38.39	AAAA
ATOM	1015	CG	ASP	109	28.062	42.354	58.800	1.00	43.78	AAAA
ATOM	1016	OD1	ASP	109	28.880	43.070	59.420	1.00	45.93	AAAA
ATOM	1017	OD2	ASP	109	27.300	41.549	59.384	1.00	47.42	AAAA
ATOM	1018	C	ASP	109	27.247	41.456	55.109	1.00	33.23	AAAA
ATOM	1019	O	ASP	109	26.350	42.237	54.827	1.00	37.51	AAAA
ATOM	1020	N	LYS	110	27.966	40.836	54.176	1.00	30.81	AAAA
ATOM	1021	H	LYS	110	28.781	40.371	54.456	1.00	0.00	AAAA
ATOM	1022	CA	LYS	110	27.619	40.913	52.747	1.00	26.81	AAAA
ATOM	1023	CB	LYS	110	28.877	40.922	51.873	1.00	26.19	AAAA
ATOM	1024	CG	LYS	110	29.898	41.966	52.267	1.00	31.88	AAAA
ATOM	1025	CD	LYS	110	31.034	42.039	51.252	1.00	35.86	AAAA

ATOM	1026	CE	LYS	110	32.009	43.165	51.577	1.00	35.07	AAAA
ATOM	1027	NZ	LYS	110	32.734	42.922	52.858	1.00	40.57	AAAA
ATOM	1028	HZ1	LYS	110	33.276	42.042	52.794	1.00	0.00	AAAA
ATOM	1029	HZ2	LYS	110	32.047	42.849	53.637	1.00	0.00	AAAA
ATOM	1030	HZ3	LYS	110	33.388	43.708	53.047	1.00	0.00	AAAA
ATOM	1031	C	LYS	110	26.772	39.722	52.335	1.00	23.39	AAAA
ATOM	1032	O	LYS	110	26.329	39.623	51.190	1.00	23.47	AAAA
ATOM	1033	N	MET	111	26.727	38.729	53.210	1.00	24.31	AAAA
ATOM	1034	H	MET	111	27.069	38.886	54.106	1.00	0.00	AAAA
ATOM	1035	CA	MET	111	26.133	37.439	52.884	1.00	24.62	AAAA
ATOM	1036	CB	MET	111	26.875	36.314	53.595	1.00	25.74	AAAA
ATOM	1037	CG	MET	111	28.355	36.281	53.307	1.00	27.29	AAAA
ATOM	1038	SD	MET	111	28.669	35.921	51.605	1.00	28.33	AAAA
ATOM	1039	CE	MET	111	29.842	37.263	51.197	1.00	22.52	AAAA
ATOM	1040	C	MET	111	24.684	37.388	53.313	1.00	25.26	AAAA
ATOM	1041	O	MET	111	24.304	37.974	54.325	1.00	24.63	AAAA
ATOM	1042	N	HIS	112	23.947	36.513	52.656	1.00	25.79	AAAA
ATOM	1043	H	HIS	112	24.366	36.111	51.894	1.00	0.00	AAAA
ATOM	1044	CA	HIS	112	22.548	36.291	52.965	1.00	27.47	AAAA
ATOM	1045	CB	HIS	112	21.683	36.549	51.723	1.00	35.41	AAAA
ATOM	1046	CG	HIS	112	20.209	36.375	51.954	1.00	42.70	AAAA
ATOM	1047	CD2	HIS	112	19.494	36.271	53.103	1.00	43.97	AAAA
ATOM	1048	ND1	HIS	112	19.300	36.261	50.924	1.00	44.16	AAAA
ATOM	1049	HD1	HIS	112	19.465	36.489	49.979	1.00	0.00	AAAA
ATOM	1050	CE1	HIS	112	18.090	36.100	51.426	1.00	46.54	AAAA
ATOM	1051	NE2	HIS	112	18.180	36.091	52.742	1.00	46.27	AAAA
ATOM	1052	HE2	HIS	112	17.442	36.197	53.384	1.00	0.00	AAAA
ATOM	1053	C	HIS	112	22.391	34.867	53.431	1.00	24.85	AAAA
ATOM	1054	O	HIS	112	22.325	33.931	52.618	1.00	23.17	AAAA
ATOM	1055	N	PHE	113	22.357	34.714	54.748	1.00	24.82	AAAA
ATOM	1056	H	PHE	113	22.542	35.493	55.300	1.00	0.00	AAAA
ATOM	1057	CA	PHE	113	22.154	33.414	55.374	1.00	29.11	AAAA
ATOM	1058	CB	PHE	113	22.758	33.401	56.794	1.00	27.84	AAAA
ATOM	1059	CG	PHE	113	24.247	33.637	56.832	1.00	24.66	AAAA
ATOM	1060	CD1	PHE	113	24.753	34.910	57.086	1.00	23.03	AAAA
ATOM	1061	CD2	PHE	113	25.141	32.586	56.598	1.00	25.31	AAAA
ATOM	1062	CE1	PHE	113	26.133	35.146	57.096	1.00	27.19	AAAA
ATOM	1063	CE2	PHE	113	26.520	32.814	56.608	1.00	24.97	AAAA
ATOM	1064	CZ	PHE	113	27.014	34.100	56.858	1.00	24.09	AAAA
ATOM	1065	C	PHE	113	20.670	33.008	55.449	1.00	31.00	AAAA
ATOM	1066	O	PHE	113	19.790	33.848	55.686	1.00	33.09	AAAA
ATOM	1067	N	GLU	114	20.402	31.772	55.043	1.00	29.35	AAAA
ATOM	1068	H	GLU	114	21.111	31.342	54.547	1.00	0.00	AAAA

ATOM	1069	CA	GLU	114	19.173	31.085	55.391	1.00	29.30 AAAA
ATOM	1070	CB	GLU	114	19.167	29.712	54.748	1.00	30.12 AAAA
ATOM	1071	CG	GLU	114	19.228	29.750	53.232	1.00	32.89 AAAA
ATOM	1072	CD	GLU	114	19.340	28.379	52.624	1.00	34.82 AAAA
ATOM	1073	OE1	GLU	114	19.097	27.384	53.338	1.00	37.36 AAAA
ATOM	1074	OE2	GLU	114	19.713	28.293	51.437	1.00	43.01 AAAA
ATOM	1075	C	GLU	114	19.067	30.927	56.903	1.00	32.03 AAAA
ATOM	1076	O	GLU	114	20.080	30.731	57.575	1.00	30.35 AAAA
ATOM	1077	N	SER	115	17.836	30.830	57.403	1.00	32.06 AAAA
ATOM	1078	H	SER	115	17.081	30.845	56.781	1.00	0.00 AAAA
ATOM	1079	CA	SER	115	17.596	30.797	58.849	1.00	34.83 AAAA
ATOM	1080	CB	SER	115	16.098	30.682	59.157	1.00	37.46 AAAA
ATOM	1081	OG	SER	115	15.337	31.576	58.368	1.00	40.20 AAAA
ATOM	1082	HG	SER	115	15.254	31.197	57.494	1.00	0.00 AAAA
ATOM	1083	C	SER	115	18.324	29.642	59.522	1.00	32.68 AAAA
ATOM	1084	O	SER	115	18.964	29.820	60.551	1.00	34.71 AAAA
ATOM	1085	N	GLY	116	18.230	28.461	58.931	1.00	28.08 AAAA
ATOM	1086	H	GLY	116	17.723	28.386	58.104	1.00	0.00 AAAA
ATOM	1087	CA	GLY	116	18.890	27.314	59.525	1.00	30.75 AAAA
ATOM	1088	C	GLY	116	20.331	27.077	59.087	1.00	29.04 AAAA
ATOM	1089	O	GLY	116	20.821	25.954	59.221	1.00	27.89 AAAA
ATOM	1090	N	SER	117	21.011	28.123	58.601	1.00	28.86 AAAA
ATOM	1091	H	SER	117	20.613	29.011	58.704	1.00	0.00 AAAA
ATOM	1092	CA	SER	117	22.365	28.000	58.017	1.00	25.44 AAAA
ATOM	1093	CB	SER	117	22.867	29.378	57.561	1.00	23.69 AAAA
ATOM	1094	OG	SER	117	24.260	29.369	57.281	1.00	21.52 AAAA
ATOM	1095	HG	SER	117	24.415	28.790	56.527	1.00	0.00 AAAA
ATOM	1096	C	SER	117	23.352	27.412	59.027	1.00	24.07 AAAA
ATOM	1097	O	SER	117	23.536	27.980	60.107	1.00	23.98 AAAA
ATOM	1098	N	THR	118	23.959	26.271	58.701	1.00	22.14 AAAA
ATOM	1099	H	THR	118	23.711	25.878	57.836	1.00	0.00 AAAA
ATOM	1100	CA	THR	118	24.950	25.660	59.593	1.00	23.25 AAAA
ATOM	1101	CB	THR	118	25.389	24.266	59.128	1.00	26.09 AAAA
ATOM	1102	OG1	THR	118	25.837	24.326	57.771	1.00	26.52 AAAA
ATOM	1103	HG1	THR	118	25.089	24.335	57.150	1.00	0.00 AAAA
ATOM	1104	CG2	THR	118	24.255	23.298	59.235	1.00	28.30 AAAA
ATOM	1105	C	THR	118	26.219	26.506	59.721	1.00	25.21 AAAA
ATOM	1106	O	THR	118	26.783	26.627	60.806	1.00	23.40 AAAA
ATOM	1107	N	LEU	119	26.662	27.117	58.623	1.00	24.77 AAAA
ATOM	1108	H	LEU	119	26.247	26.892	57.763	1.00	0.00 AAAA
ATOM	1109	CA	LEU	119	27.793	28.025	58.711	1.00	21.55 AAAA
ATOM	1110	CB	LEU	119	28.278	28.446	57.317	1.00	21.35 AAAA
ATOM	1111	CG	LEU	119	29.527	29.346	57.292	1.00	22.12 AAAA

ATOM	1112	CD1	LEU	119	30.675	28.670	58.031	1.00	21.90	AAAA
ATOM	1113	CD2	LEU	119	29.938	29.618	55.852	1.00	25.56	AAAA
ATOM	1114	C	LEU	119	27.447	29.257	59.547	1.00	22.56	AAAA
ATOM	1115	O	LEU	119	28.249	29.680	60.377	1.00	21.20	AAAA
ATOM	1116	N	LYS	120	26.240	29.800	59.384	1.00	19.77	AAAA
ATOM	1117	H	LYS	120	25.624	29.429	58.719	1.00	0.00	AAAA
ATOM	1118	CA	LYS	120	25.847	30.974	60.164	1.00	20.58	AAAA
ATOM	1119	CB	LYS	120	24.408	31.368	59.844	1.00	27.26	AAAA
ATOM	1120	CG	LYS	120	23.917	32.629	60.548	1.00	31.17	AAAA
ATOM	1121	CD	LYS	120	22.398	32.703	60.494	1.00	37.84	AAAA
ATOM	1122	CE	LYS	120	21.870	33.951	61.161	1.00	41.70	AAAA
ATOM	1123	NZ	LYS	120	22.115	33.910	62.630	1.00	46.53	AAAA
ATOM	1124	HZ1	LYS	120	23.142	33.971	62.791	1.00	0.00	AAAA
ATOM	1125	HZ2	LYS	120	21.749	33.022	63.030	1.00	0.00	AAAA
ATOM	1126	HZ3	LYS	120	21.652	34.725	63.081	1.00	0.00	AAAA
ATOM	1127	C	LYS	120	25.978	30.687	61.659	1.00	21.21	AAAA
ATOM	1128	O	LYS	120	26.554	31.485	62.406	1.00	21.15	AAAA
ATOM	1129	N	LYS	121	25.509	29.511	62.066	1.00	21.91	AAAA
ATOM	1130	H	LYS	121	25.104	28.933	61.385	1.00	0.00	AAAA
ATOM	1131	CA	LYS	121	25.564	29.109	63.460	1.00	22.16	AAAA
ATOM	1132	CB	LYS	121	24.764	27.828	63.710	1.00	22.21	AAAA
ATOM	1133	CG	LYS	121	24.645	27.532	65.204	1.00	26.49	AAAA
ATOM	1134	CD	LYS	121	24.221	26.114	65.506	1.00	30.08	AAAA
ATOM	1135	CE	LYS	121	24.099	25.911	67.019	1.00	32.98	AAAA
ATOM	1136	NZ	LYS	121	23.937	24.481	67.402	1.00	34.13	AAAA
ATOM	1137	HZ1	LYS	121	24.516	23.891	66.772	1.00	0.00	AAAA
ATOM	1138	HZ2	LYS	121	22.943	24.188	67.309	1.00	0.00	AAAA
ATOM	1139	HZ3	LYS	121	24.240	24.348	68.384	1.00	0.00	AAAA
ATOM	1140	C	LYS	121	26.994	28.898	63.946	1.00	23.88	AAAA
ATOM	1141	O	LYS	121	27.315	29.236	65.088	1.00	24.24	AAAA
ATOM	1142	N	PHE	122	27.834	28.301	63.104	1.00	21.06	AAAA
ATOM	1143	H	PHE	122	27.476	27.916	62.273	1.00	0.00	AAAA
ATOM	1144	CA	PHE	122	29.258	28.160	63.437	1.00	20.87	AAAA
ATOM	1145	CB	PHE	122	30.001	27.412	62.333	1.00	18.89	AAAA
ATOM	1146	CG	PHE	122	31.468	27.264	62.599	1.00	21.64	AAAA
ATOM	1147	CD1	PHE	122	31.950	26.174	63.324	1.00	21.50	AAAA
ATOM	1148	CD2	PHE	122	32.369	28.255	62.192	1.00	21.09	AAAA
ATOM	1149	CE1	PHE	122	33.314	26.065	63.644	1.00	18.48	AAAA
ATOM	1150	CE2	PHE	122	33.727	28.158	62.515	1.00	19.92	AAAA
ATOM	1151	CZ	PHE	122	34.192	27.058	63.239	1.00	18.04	AAAA
ATOM	1152	C	PHE	122	29.934	29.514	63.670	1.00	20.43	AAAA
ATOM	1153	O	PHE	122	30.666	29.699	64.647	1.00	22.71	AAAA
ATOM	1154	N	LEU	123	29.646	30.471	62.800	1.00	16.51	AAAA

ATOM	1155	H	LEU	123	29.032	30.251	62.069	1.00	0.00	AAAA
ATOM	1156	CA	LEU	123	30.224	31.793	62.905	1.00	19.43	AAAA
ATOM	1157	CB	LEU	123	29.913	32.592	61.645	1.00	16.02	AAAA
ATOM	1158	CG	LEU	123	30.559	32.020	60.383	1.00	15.88	AAAA
ATOM	1159	CD1	LEU	123	30.139	32.849	59.185	1.00	16.91	AAAA
ATOM	1160	CD2	LEU	123	32.097	32.005	60.562	1.00	17.98	AAAA
ATOM	1161	C	LEU	123	29.777	32.574	64.137	1.00	23.21	AAAA
ATOM	1162	O	LEU	123	30.581	33.249	64.777	1.00	22.18	AAAA
ATOM	1163	N	GLU	124	28.492	32.499	64.469	1.00	24.39	AAAA
ATOM	1164	H	GLU	124	27.889	31.989	63.882	1.00	0.00	AAAA
ATOM	1165	CA	GLU	124	28.003	33.237	65.628	1.00	25.74	AAAA
ATOM	1166	CB	GLU	124	26.474	33.412	65.587	1.00	29.50	AAAA
ATOM	1167	CG	GLU	124	25.678	32.166	65.281	1.00	36.33	AAAA
ATOM	1168	CD	GLU	124	24.262	32.483	64.795	1.00	42.27	AAAA
ATOM	1169	OE1	GLU	124	23.941	33.685	64.630	1.00	43.67	AAAA
ATOM	1170	OE2	GLU	124	23.474	31.531	64.578	1.00	42.51	AAAA
ATOM	1171	C	GLU	124	28.439	32.576	66.924	1.00	21.63	AAAA
ATOM	1172	O	GLU	124	28.850	33.260	67.850	1.00	25.47	AAAA
ATOM	1173	N	GLU	125	28.531	31.256	66.928	1.00	20.31	AAAA
ATOM	1174	H	GLU	125	28.213	30.752	66.151	1.00	0.00	AAAA
ATOM	1175	CA	GLU	125	29.072	30.570	68.091	1.00	24.49	AAAA
ATOM	1176	CB	GLU	125	28.836	29.061	68.010	1.00	29.56	AAAA
ATOM	1177	CG	GLU	125	27.352	28.625	68.085	1.00	35.07	AAAA
ATOM	1178	CD	GLU	125	26.599	29.184	69.303	1.00	40.25	AAAA
ATOM	1179	OE1	GLU	125	27.239	29.516	70.334	1.00	38.36	AAAA
ATOM	1180	OE2	GLU	125	25.353	29.280	69.224	1.00	40.90	AAAA
ATOM	1181	C	GLU	125	30.558	30.832	68.290	1.00	25.02	AAAA
ATOM	1182	O	GLU	125	31.037	30.829	69.426	1.00	27.15	AAAA
ATOM	1183	N	SER	126	31.291	31.034	67.194	1.00	24.83	AAAA
ATOM	1184	H	SER	126	30.820	31.102	66.338	1.00	0.00	AAAA
ATOM	1185	CA	SER	126	32.759	31.135	67.256	1.00	22.04	AAAA
ATOM	1186	CB	SER	126	33.406	30.320	66.130	1.00	19.89	AAAA
ATOM	1187	OG	SER	126	33.126	30.932	64.880	1.00	18.20	AAAA
ATOM	1188	HG	SER	126	32.193	30.782	64.685	1.00	0.00	AAAA
ATOM	1189	C	SER	126	33.294	32.562	67.200	1.00	23.67	AAAA
ATOM	1190	O	SER	126	34.507	32.768	67.080	1.00	25.05	AAAA
ATOM	1191	N	VAL	127	32.422	33.533	67.456	1.00	22.04	AAAA
ATOM	1192	H	VAL	127	31.518	33.298	67.748	1.00	0.00	AAAA
ATOM	1193	CA	VAL	127	32.757	34.942	67.278	1.00	24.22	AAAA
ATOM	1194	CB	VAL	127	31.479	35.822	67.390	1.00	25.46	AAAA
ATOM	1195	CG1	VAL	127	31.117	36.056	68.842	1.00	27.14	AAAA
ATOM	1196	CG2	VAL	127	31.667	37.136	66.669	1.00	29.08	AAAA
ATOM	1197	C	VAL	127	33.817	35.436	68.274	1.00	25.27	AAAA

ATOM	1198	O	VAL	127	34.596	36.339	67.977	1.00	26.45	AAAA
ATOM	1199	N	SER	128	33.872	34.807	69.440	1.00	25.75	AAAA
ATOM	1200	H	SER	128	33.200	34.110	69.594	1.00	0.00	AAAA
ATOM	1201	CA	SER	128	34.781	35.242	70.497	1.00	26.71	AAAA
ATOM	1202	CB	SER	128	33.983	35.692	71.723	1.00	26.97	AAAA
ATOM	1203	OG	SER	128	33.345	36.937	71.487	1.00	32.77	AAAA
ATOM	1204	HG	SER	128	33.932	37.594	71.090	1.00	0.00	AAAA
ATOM	1205	C	SER	128	35.786	34.166	70.909	1.00	27.80	AAAA
ATOM	1206	O	SER	128	36.560	34.351	71.857	1.00	29.12	AAAA
ATOM	1207	N	MET	129	35.807	33.056	70.180	1.00	22.86	AAAA
ATOM	1208	H	MET	129	35.182	33.002	69.438	1.00	0.00	AAAA
ATOM	1209	CA	MET	129	36.810	32.030	70.416	1.00	20.85	AAAA
ATOM	1210	CB	MET	129	36.426	30.738	69.710	1.00	21.01	AAAA
ATOM	1211	CG	MET	129	35.109	30.156	70.115	1.00	21.58	AAAA
ATOM	1212	SD	MET	129	34.770	28.650	69.190	1.00	24.74	AAAA
ATOM	1213	CE	MET	129	33.164	28.188	69.880	1.00	20.30	AAAA
ATOM	1214	C	MET	129	38.166	32.500	69.875	1.00	21.66	AAAA
ATOM	1215	O	MET	129	38.238	33.329	68.961	1.00	19.22	AAAA
ATOM	1216	N	SER	130	39.232	31.880	70.361	1.00	20.89	AAAA
ATOM	1217	H	SER	130	39.092	31.193	71.016	1.00	0.00	AAAA
ATOM	1218	CA	SER	130	40.568	32.109	69.800	1.00	21.92	AAAA
ATOM	1219	CB	SER	130	41.633	31.625	70.783	1.00	13.02	AAAA
ATOM	1220	OG	SER	130	41.639	30.219	70.811	1.00	14.33	AAAA
ATOM	1221	HG	SER	130	42.205	29.949	71.540	1.00	0.00	AAAA
ATOM	1222	C	SER	130	40.726	31.351	68.464	1.00	20.98	AAAA
ATOM	1223	O	SER	130	40.168	30.269	68.293	1.00	22.57	AAAA
ATOM	1224	N	PRO	131	41.686	31.774	67.624	1.00	21.58	AAAA
ATOM	1225	CD	PRO	131	42.485	33.009	67.725	1.00	18.87	AAAA
ATOM	1226	CA	PRO	131	42.056	30.997	66.431	1.00	19.82	AAAA
ATOM	1227	CB	PRO	131	43.341	31.675	65.975	1.00	18.16	AAAA
ATOM	1228	CG	PRO	131	43.131	33.097	66.364	1.00	18.91	AAAA
ATOM	1229	C	PRO	131	42.256	29.503	66.685	1.00	18.54	AAAA
ATOM	1230	O	PRO	131	41.773	28.660	65.939	1.00	19.61	AAAA
ATOM	1231	N	GLU	132	42.866	29.180	67.811	1.00	17.68	AAAA
ATOM	1232	H	GLU	132	43.096	29.935	68.378	1.00	0.00	AAAA
ATOM	1233	CA	GLU	132	43.061	27.789	68.210	1.00	20.89	AAAA
ATOM	1234	CB	GLU	132	44.060	27.717	69.377	1.00	21.09	AAAA
ATOM	1235	CG	GLU	132	45.413	28.416	69.127	1.00	32.04	AAAA
ATOM	1236	CD	GLU	132	45.333	29.949	69.017	1.00	36.37	AAAA
ATOM	1237	OE1	GLU	132	44.489	30.566	69.701	1.00	37.91	AAAA
ATOM	1238	OE2	GLU	132	46.148	30.546	68.269	1.00	42.82	AAAA
ATOM	1239	C	GLU	132	41.727	27.114	68.620	1.00	17.56	AAAA
ATOM	1240	O	GLU	132	41.441	25.973	68.252	1.00	18.36	AAAA

ATOM	1241	N	GLU	133	40.913	27.830	69.380	1.00	20.52	AAAA
ATOM	1242	H	GLU	133	41.184	28.735	69.645	1.00	0.00	AAAA
ATOM	1243	CA	GLU	133	39.625	27.290	69.796	1.00	20.73	AAAA
ATOM	1244	CB	GLU	133	38.943	28.259	70.762	1.00	23.60	AAAA
ATOM	1245	CG	GLU	133	39.479	28.128	72.198	1.00	24.26	AAAA
ATOM	1246	CD	GLU	133	39.027	29.241	73.118	1.00	25.00	AAAA
ATOM	1247	OE1	GLU	133	38.859	30.389	72.659	1.00	25.09	AAAA
ATOM	1248	OE2	GLU	133	38.907	28.977	74.331	1.00	28.62	AAAA
ATOM	1249	C	GLU	133	38.730	27.019	68.597	1.00	18.69	AAAA
ATOM	1250	O	GLU	133	38.093	25.967	68.514	1.00	19.64	AAAA
ATOM	1251	N	ARG	134	38.800	27.908	67.612	1.00	20.05	AAAA
ATOM	1252	H	ARG	134	39.396	28.673	67.722	1.00	0.00	AAAA
ATOM	1253	CA	ARG	134	37.986	27.786	66.391	1.00	18.25	AAAA
ATOM	1254	CB	ARG	134	38.135	29.038	65.543	1.00	13.07	AAAA
ATOM	1255	CG	ARG	134	37.576	30.266	66.200	1.00	12.02	AAAA
ATOM	1256	CD	ARG	134	37.921	31.498	65.435	1.00	14.57	AAAA
ATOM	1257	NE	ARG	134	37.128	32.629	65.891	1.00	14.70	AAAA
ATOM	1258	HE	ARG	134	36.251	32.439	66.282	1.00	0.00	AAAA
ATOM	1259	CZ	ARG	134	37.518	33.894	65.804	1.00	17.08	AAAA
ATOM	1260	NH1	ARG	134	36.702	34.867	66.202	1.00	19.69	AAAA
ATOM	1261	HH11	ARG	134	37.003	35.819	66.165	1.00	0.00	AAAA
ATOM	1262	HH12	ARG	134	35.791	34.643	66.549	1.00	0.00	AAAA
ATOM	1263	NH2	ARG	134	38.697	34.191	65.267	1.00	17.97	AAAA
ATOM	1264	HH21	ARG	134	39.284	33.466	64.908	1.00	0.00	AAAA
ATOM	1265	HH22	ARG	134	38.996	35.145	65.226	1.00	0.00	AAAA
ATOM	1266	C	ARG	134	38.311	26.543	65.566	1.00	17.76	AAAA
ATOM	1267	O	ARG	134	37.406	25.854	65.078	1.00	17.79	AAAA
ATOM	1268	N	ALA	135	39.585	26.156	65.568	1.00	16.35	AAAA
ATOM	1269	H	ALA	135	40.235	26.734	66.017	1.00	0.00	AAAA
ATOM	1270	CA	ALA	135	40.009	24.934	64.905	1.00	16.21	AAAA
ATOM	1271	CB	ALA	135	41.538	24.867	64.833	1.00	15.84	AAAA
ATOM	1272	C	ALA	135	39.462	23.712	65.635	1.00	19.65	AAAA
ATOM	1273	O	ALA	135	39.029	22.744	65.010	1.00	20.84	AAAA
ATOM	1274	N	ARG	136	39.476	23.762	66.963	1.00	21.92	AAAA
ATOM	1275	H	ARG	136	39.873	24.554	67.393	1.00	0.00	AAAA
ATOM	1276	CA	ARG	136	38.935	22.677	67.785	1.00	22.78	AAAA
ATOM	1277	CB	ARG	136	39.268	22.948	69.255	1.00	22.88	AAAA
ATOM	1278	CG	ARG	136	39.719	21.737	70.028	1.00	30.16	AAAA
ATOM	1279	CD	ARG	136	40.153	22.137	71.432	0.00	26.92	AAAA
ATOM	1280	NE	ARG	136	40.848	21.059	72.132	0.00	27.01	AAAA
ATOM	1281	HE	ARG	136	41.821	20.997	72.030	0.00	0.00	AAAA
ATOM	1282	CZ	ARG	136	40.251	20.159	72.908	0.00	26.44	AAAA
ATOM	1283	NH1	ARG	136	40.975	19.250	73.546	0.00	26.25	AAAA

ATOM	1284	HH11	ARG	136	41.972	19.258	73.462	1.00	0.00	AAAA
ATOM	1285	HH12	ARG	136	40.527	18.592	74.150	1.00	0.00	AAAA
ATOM	1286	NH2	ARG	136	38.930	20.142	73.022	0.00	26.23	AAAA
ATOM	1287	HH21	ARG	136	38.372	20.799	72.513	1.00	0.00	AAAA
ATOM	1288	HH22	ARG	136	38.488	19.468	73.613	1.00	0.00	AAAA
ATOM	1289	C	ARG	136	37.400	22.548	67.608	1.00	20.95	AAAA
ATOM	1290	O	ARG	136	36.854	21.445	67.486	1.00	20.97	AAAA
ATOM	1291	N	TYR	137	36.717	23.684	67.612	1.00	17.84	AAAA
ATOM	1292	H	TYR	137	37.205	24.511	67.800	1.00	0.00	AAAA
ATOM	1293	CA	TYR	137	35.289	23.702	67.369	1.00	17.54	AAAA
ATOM	1294	CB	TYR	137	34.772	25.129	67.536	1.00	18.25	AAAA
ATOM	1295	CG	TYR	137	33.262	25.292	67.445	1.00	21.44	AAAA
ATOM	1296	CD1	TYR	137	32.388	24.288	67.867	1.00	22.83	AAAA
ATOM	1297	CE1	TYR	137	30.989	24.460	67.767	1.00	24.57	AAAA
ATOM	1298	CD2	TYR	137	32.712	26.467	66.931	1.00	24.44	AAAA
ATOM	1299	CE2	TYR	137	31.336	26.645	66.837	1.00	23.60	AAAA
ATOM	1300	CZ	TYR	137	30.481	25.645	67.253	1.00	23.28	AAAA
ATOM	1301	OH	TYR	137	29.124	25.869	67.158	1.00	26.64	AAAA
ATOM	1302	HH	TYR	137	28.634	25.119	67.487	1.00	0.00	AAAA
ATOM	1303	C	TYR	137	34.982	23.136	65.969	1.00	19.09	AAAA
ATOM	1304	O	TYR	137	34.284	22.126	65.862	1.00	19.43	AAAA
ATOM	1305	N	LEU	138	35.682	23.621	64.939	1.00	18.53	AAAA
ATOM	1306	H	LEU	138	36.295	24.357	65.103	1.00	0.00	AAAA
ATOM	1307	CA	LEU	138	35.518	23.068	63.582	1.00	20.95	AAAA
ATOM	1308	CB	LEU	138	36.472	23.730	62.586	1.00	17.16	AAAA
ATOM	1309	CG	LEU	138	36.270	23.242	61.144	1.00	17.24	AAAA
ATOM	1310	CD1	LEU	138	34.887	23.658	60.627	1.00	18.51	AAAA
ATOM	1311	CD2	LEU	138	37.357	23.808	60.262	1.00	18.65	AAAA
ATOM	1312	C	LEU	138	35.679	21.547	63.480	1.00	23.49	AAAA
ATOM	1313	O	LEU	138	34.883	20.888	62.811	1.00	23.89	AAAA
ATOM	1314	N	GLU	139	36.717	21.001	64.115	1.00	23.27	AAAA
ATOM	1315	H	GLU	139	37.378	21.603	64.525	1.00	0.00	AAAA
ATOM	1316	CA	GLU	139	36.884	19.555	64.225	1.00	26.59	AAAA
ATOM	1317	CB	GLU	139	38.111	19.221	65.081	1.00	31.75	AAAA
ATOM	1318	CG	GLU	139	39.429	19.788	64.559	1.00	42.46	AAAA
ATOM	1319	CD	GLU	139	40.623	19.513	65.491	1.00	47.40	AAAA
ATOM	1320	OE1	GLU	139	41.176	18.390	65.443	1.00	50.41	AAAA
ATOM	1321	OE2	GLU	139	41.027	20.429	66.245	1.00	50.25	AAAA
ATOM	1322	C	GLU	139	35.639	18.897	64.842	1.00	27.54	AAAA
ATOM	1323	O	GLU	139	35.147	17.903	64.318	1.00	30.37	AAAA
ATOM	1324	N	ASN	140	35.090	19.484	65.904	1.00	26.57	AAAA
ATOM	1325	H	ASN	140	35.529	20.277	66.280	1.00	0.00	AAAA
ATOM	1326	CA	ASN	140	33.884	18.922	66.545	1.00	30.15	AAAA

ATOM	1327	CB	ASN	140	33.647	19.558	67.922	1.00	32.80	AAAA
ATOM	1328	CG	ASN	140	34.831	19.416	68.854	1.00	35.71	AAAA
ATOM	1329	OD1	ASN	140	35.740	18.621	68.634	1.00	37.11	AAAA
ATOM	1330	ND2	ASN	140	34.828	20.210	69.906	1.00	38.04	AAAA
ATOM	1331	HD21	ASN	140	34.054	20.795	70.059	1.00	0.00	AAAA
ATOM	1332	HD22	ASN	140	35.627	20.136	70.454	1.00	0.00	AAAA
ATOM	1333	C	ASN	140	32.601	19.106	65.720	1.00	30.97	AAAA
ATOM	1334	O	ASN	140	31.654	18.317	65.819	1.00	33.89	AAAA
ATOM	1335	N	TYR	141	32.524	20.242	65.041	1.00	29.95	AAAA
ATOM	1336	H	TYR	141	33.363	20.717	64.917	1.00	0.00	AAAA
ATOM	1337	CA	TYR	141	31.299	20.738	64.430	1.00	28.02	AAAA
ATOM	1338	CB	TYR	141	31.466	22.222	64.099	1.00	25.30	AAAA
ATOM	1339	CG	TYR	141	30.191	22.921	63.727	1.00	23.02	AAAA
ATOM	1340	CD1	TYR	141	29.318	23.352	64.711	1.00	24.68	AAAA
ATOM	1341	CE1	TYR	141	28.155	24.034	64.396	1.00	23.87	AAAA
ATOM	1342	CD2	TYR	141	29.878	23.186	62.400	1.00	17.67	AAAA
ATOM	1343	CE2	TYR	141	28.722	23.874	62.073	1.00	26.80	AAAA
ATOM	1344	CZ	TYR	141	27.863	24.298	63.083	1.00	25.22	AAAA
ATOM	1345	OH	TYR	141	26.738	25.024	62.790	1.00	27.16	AAAA
ATOM	1346	HH	TYR	141	26.724	25.168	61.847	1.00	0.00	AAAA
ATOM	1347	C	TYR	141	30.968	19.958	63.162	1.00	29.46	AAAA
ATOM	1348	O	TYR	141	31.220	20.422	62.047	1.00	28.34	AAAA
ATOM	1349	N	ASP	142	30.217	18.882	63.349	1.00	30.05	AAAA
ATOM	1350	H	ASP	142	29.913	18.788	64.276	1.00	0.00	AAAA
ATOM	1351	CA	ASP	142	29.980	17.888	62.311	1.00	33.85	AAAA
ATOM	1352	CB	ASP	142	29.123	16.750	62.880	1.00	42.00	AAAA
ATOM	1353	CG	ASP	142	29.400	16.473	64.361	1.00	49.95	AAAA
ATOM	1354	OD1	ASP	142	28.696	17.052	65.230	1.00	48.78	AAAA
ATOM	1355	OD2	ASP	142	30.288	15.633	64.648	1.00	53.91	AAAA
ATOM	1356	C	ASP	142	29.308	18.452	61.047	1.00	32.97	AAAA
ATOM	1357	O	ASP	142	29.589	18.017	59.926	1.00	31.12	AAAA
ATOM	1358	N	ALA	143	28.443	19.442	61.243	1.00	30.82	AAAA
ATOM	1359	H	ALA	143	28.396	19.832	62.132	1.00	0.00	AAAA
ATOM	1360	CA	ALA	143	27.622	19.997	60.176	1.00	31.33	AAAA
ATOM	1361	CB	ALA	143	26.685	21.056	60.747	1.00	30.92	AAAA
ATOM	1362	C	ALA	143	28.407	20.577	58.989	1.00	32.79	AAAA
ATOM	1363	O	ALA	143	27.900	20.600	57.869	1.00	37.58	AAAA
ATOM	1364	N	ILE	144	29.628	21.061	59.215	1.00	29.28	AAAA
ATOM	1365	H	ILE	144	30.015	21.012	60.119	1.00	0.00	AAAA
ATOM	1366	CA	ILE	144	30.416	21.591	58.107	1.00	27.01	AAAA
ATOM	1367	CB	ILE	144	31.344	22.736	58.568	1.00	27.54	AAAA
ATOM	1368	CG2	ILE	144	32.231	23.223	57.416	1.00	27.82	AAAA
ATOM	1369	CG1	ILE	144	30.485	23.919	59.024	1.00	25.26	AAAA

ATOM	1370	CD	ILE	144	31.254	25.121	59.464	1.00	24.22	AAAA
ATOM	1371	C	ILE	144	31.177	20.463	57.420	1.00	29.24	AAAA
ATOM	1372	O	ILE	144	32.313	20.126	57.774	1.00	30.95	AAAA
ATOM	1373	N	ARG	145	30.454	19.795	56.524	1.00	28.91	AAAA
ATOM	1374	H	ARG	145	29.547	20.114	56.340	1.00	0.00	AAAA
ATOM	1375	CA	ARG	145	30.895	18.579	55.834	1.00	29.33	AAAA
ATOM	1376	CB	ARG	145	30.311	17.342	56.539	1.00	30.28	AAAA
ATOM	1377	CG	ARG	145	29.988	16.150	55.646	0.00	29.38	AAAA
ATOM	1378	CD	ARG	145	31.078	15.091	55.690	0.00	29.04	AAAA
ATOM	1379	NE	ARG	145	31.399	14.683	57.056	0.00	28.60	AAAA
ATOM	1380	HE	ARG	145	31.308	15.336	57.782	0.00	0.00	AAAA
ATOM	1381	CZ	ARG	145	31.833	13.473	57.396	0.00	28.42	AAAA
ATOM	1382	NH1	ARG	145	32.122	13.208	58.663	0.00	28.25	AAAA
ATOM	1383	HH11	ARG	145	32.021	13.923	59.354	1.00	0.00	AAAA
ATOM	1384	HH12	ARG	145	32.454	12.300	58.923	1.00	0.00	AAAA
ATOM	1385	NH2	ARG	145	31.974	12.524	56.479	0.00	28.30	AAAA
ATOM	1386	HH21	ARG	145	32.314	11.623	56.749	1.00	0.00	AAAA
ATOM	1387	HH22	ARG	145	31.721	12.696	55.526	1.00	0.00	AAAA
ATOM	1388	C	ARG	145	30.355	18.676	54.415	1.00	29.88	AAAA
ATOM	1389	O	ARG	145	29.256	19.167	54.236	1.00	31.53	AAAA
ATOM	1390	N	VAL	146	31.187	18.416	53.415	1.00	31.91	AAAA
ATOM	1391	H	VAL	146	32.098	18.132	53.593	1.00	0.00	AAAA
ATOM	1392	CA	VAL	146	30.698	18.434	52.042	1.00	37.37	AAAA
ATOM	1393	CB	VAL	146	31.845	18.531	50.978	1.00	36.83	AAAA
ATOM	1394	CG1	VAL	146	32.686	19.774	51.208	1.00	33.88	AAAA
ATOM	1395	CG2	VAL	146	32.717	17.285	50.996	1.00	36.91	AAAA
ATOM	1396	C	VAL	146	29.899	17.155	51.815	1.00	44.69	AAAA
ATOM	1397	OT1	VAL	146	30.301	16.119	52.395	1.00	50.73	AAAA
ATOM	1398	OT2	VAL	146	28.808	17.228	51.203	1.00	51.33	AAAA
ATOM	1399	CB	ASP	167	51.716	18.609	47.898	1.00	49.54	BBBB
ATOM	1400	CG	ASP	167	53.148	18.936	47.435	1.00	55.49	BBBB
ATOM	1401	OD1	ASP	167	53.852	17.986	47.033	1.00	58.95	BBBB
ATOM	1402	OD2	ASP	167	53.560	20.123	47.426	1.00	57.98	BBBB
ATOM	1403	C	ASP	167	50.546	20.690	48.673	1.00	38.29	BBBB
ATOM	1404	O	ASP	167	51.079	21.780	48.786	1.00	37.35	BBBB
ATOM	1405	HT1	ASP	167	49.531	18.285	49.508	1.00	0.00	BBBB
ATOM	1406	HT2	ASP	167	50.055	19.203	50.836	1.00	0.00	BBBB
ATOM	1407	N	ASP	167	50.385	18.623	50.034	1.00	44.61	BBBB
ATOM	1408	HT3	ASP	167	50.902	17.811	50.403	1.00	0.00	BBBB
ATOM	1409	CA	ASP	167	51.272	19.431	49.130	1.00	43.35	BBBB
ATOM	1410	N	LEU	168	49.330	20.523	48.152	1.00	32.28	BBBB
ATOM	1411	H	LEU	168	48.993	19.646	47.910	1.00	0.00	BBBB
ATOM	1412	CA	LEU	168	48.483	21.649	47.735	1.00	30.48	BBBB

ATOM	1413	CB	LEU	168	47.434	21.174	46.711	1.00	29.61	BBBB
ATOM	1414	CG	LEU	168	47.519	21.629	45.234	1.00	34.32	BBBB
ATOM	1415	CD1	LEU	168	48.957	21.832	44.759	1.00	27.65	BBBB
ATOM	1416	CD2	LEU	168	46.800	20.592	44.368	1.00	27.35	BBBB
ATOM	1417	C	LEU	168	47.786	22.321	48.937	1.00	27.15	BBBB
ATOM	1418	O	LEU	168	47.365	21.644	49.867	1.00	28.57	BBBB
ATOM	1419	N	HIS	169	47.712	23.649	48.927	1.00	22.41	BBBB
ATOM	1420	H	HIS	169	47.989	24.120	48.109	1.00	0.00	BBBB
ATOM	1421	CA	HIS	169	47.223	24.415	50.068	1.00	16.79	BBBB
ATOM	1422	CB	HIS	169	48.425	24.912	50.889	1.00	15.93	BBBB
ATOM	1423	CG	HIS	169	48.067	25.462	52.235	1.00	13.63	BBBB
ATOM	1424	CD2	HIS	169	48.301	26.674	52.792	1.00	13.31	BBBB
ATOM	1425	ND1	HIS	169	47.275	24.780	53.135	1.00	19.04	BBBB
ATOM	1426	HD1	HIS	169	46.915	23.885	53.069	1.00	0.00	BBBB
ATOM	1427	CE1	HIS	169	47.024	25.550	54.177	1.00	15.56	BBBB
ATOM	1428	NE2	HIS	169	47.641	26.704	53.996	1.00	14.61	BBBB
ATOM	1429	HE2	HIS	169	47.457	27.477	54.543	1.00	0.00	BBBB
ATOM	1430	C	HIS	169	46.378	25.605	49.589	1.00	15.63	BBBB
ATOM	1431	O	HIS	169	46.655	26.195	48.551	1.00	15.56	BBBB
ATOM	1432	N	PHE	170	45.336	25.936	50.347	1.00	11.37	BBBB
ATOM	1433	H	PHE	170	45.080	25.342	51.078	1.00	0.00	BBBB
ATOM	1434	CA	PHE	170	44.529	27.119	50.091	1.00	13.04	BBBB
ATOM	1435	CB	PHE	170	43.034	26.820	50.263	1.00	15.00	BBBB
ATOM	1436	CG	PHE	170	42.363	26.294	49.024	1.00	13.40	BBBB
ATOM	1437	CD1	PHE	170	42.008	24.954	48.930	1.00	16.80	BBBB
ATOM	1438	CD2	PHE	170	41.975	27.160	48.016	1.00	17.90	BBBB
ATOM	1439	CE1	PHE	170	41.253	24.481	47.862	1.00	15.73	BBBB
ATOM	1440	CE2	PHE	170	41.216	26.692	46.931	1.00	19.11	BBBB
ATOM	1441	CZ	PHE	170	40.856	25.352	46.866	1.00	14.95	BBBB
ATOM	1442	C	PHE	170	44.870	28.218	51.068	1.00	12.35	BBBB
ATOM	1443	O	PHE	170	45.009	27.977	52.273	1.00	13.04	BBBB
ATOM	1444	N	ILE	171	44.908	29.438	50.562	1.00	13.08	BBBB
ATOM	1445	H	ILE	171	44.992	29.531	49.589	1.00	0.00	BBBB
ATOM	1446	CA	ILE	171	44.790	30.602	51.423	1.00	17.13	BBBB
ATOM	1447	CB	ILE	171	46.115	31.418	51.506	1.00	17.52	BBBB
ATOM	1448	CG2	ILE	171	47.216	30.540	52.100	1.00	18.45	BBBB
ATOM	1449	CG1	ILE	171	46.520	31.954	50.133	1.00	19.39	BBBB
ATOM	1450	CD	ILE	171	47.653	32.960	50.180	1.00	21.31	BBBB
ATOM	1451	C	ILE	171	43.646	31.499	50.942	1.00	15.82	BBBB
ATOM	1452	O	ILE	171	43.136	31.317	49.826	1.00	15.55	BBBB
ATOM	1453	N	ALA	172	43.153	32.332	51.855	1.00	14.30	BBBB
ATOM	1454	H	ALA	172	43.513	32.279	52.767	1.00	0.00	BBBB
ATOM	1455	CA	ALA	172	42.126	33.309	51.554	1.00	11.34	BBBB

ATOM	1456	CB	ALA	172	40.961	33.159	52.534	1.00	12.21	BBBB
ATOM	1457	C	ALA	172	42.715	34.705	51.626	1.00	13.20	BBBB
ATOM	1458	O	ALA	172	43.537	35.009	52.500	1.00	13.74	BBBB
ATOM	1459	N	LEU	173	42.324	35.544	50.670	1.00	14.10	BBBB
ATOM	1460	H	LEU	173	41.732	35.187	49.978	1.00	0.00	BBBB
ATOM	1461	CA	LEU	173	42.744	36.942	50.630	1.00	13.28	BBBB
ATOM	1462	CB	LEU	173	43.535	37.208	49.345	1.00	14.89	BBBB
ATOM	1463	CG	LEU	173	44.958	36.611	49.358	1.00	17.25	BBBB
ATOM	1464	CD1	LEU	173	45.526	36.424	47.940	1.00	14.54	BBBB
ATOM	1465	CD2	LEU	173	45.847	37.539	50.191	1.00	15.04	BBBB
ATOM	1466	C	LEU	173	41.502	37.827	50.688	1.00	16.11	BBBB
ATOM	1467	O	LEU	173	40.568	37.637	49.907	1.00	16.45	BBBB
ATOM	1468	N	VAL	174	41.425	38.646	51.736	1.00	16.31	BBBB
ATOM	1469	H	VAL	174	42.209	38.693	52.325	1.00	0.00	BBBB
ATOM	1470	CA	VAL	174	40.237	39.448	52.028	1.00	17.92	BBBB
ATOM	1471	CB	VAL	174	39.351	38.822	53.157	1.00	17.86	BBBB
ATOM	1472	CG1	VAL	174	38.869	37.422	52.764	1.00	15.30	BBBB
ATOM	1473	CG2	VAL	174	40.130	38.787	54.480	1.00	16.97	BBBB
ATOM	1474	C	VAL	174	40.588	40.865	52.459	1.00	18.53	BBBB
ATOM	1475	O	VAL	174	41.690	41.149	52.931	1.00	17.41	BBBB
ATOM	1476	N	HIS	175	39.623	41.751	52.294	1.00	18.01	BBBB
ATOM	1477	H	HIS	175	38.768	41.427	51.928	1.00	0.00	BBBB
ATOM	1478	CA	HIS	175	39.784	43.137	52.667	1.00	22.89	BBBB
ATOM	1479	CB	HIS	175	39.178	44.038	51.590	1.00	24.46	BBBB
ATOM	1480	CG	HIS	175	38.940	45.443	52.047	1.00	28.42	BBBB
ATOM	1481	CD2	HIS	175	39.797	46.404	52.459	1.00	29.90	BBBB
ATOM	1482	ND1	HIS	175	37.680	45.987	52.146	1.00	29.90	BBBB
ATOM	1483	HD1	HIS	175	36.868	45.469	51.941	1.00	0.00	BBBB
ATOM	1484	CE1	HIS	175	37.769	47.222	52.596	1.00	30.93	BBBB
ATOM	1485	NE2	HIS	175	39.044	47.499	52.797	1.00	31.81	BBBB
ATOM	1486	HE2	HIS	175	39.397	48.399	52.952	1.00	0.00	BBBB
ATOM	1487	C	HIS	175	39.090	43.373	53.994	1.00	23.68	BBBB
ATOM	1488	O	HIS	175	37.890	43.165	54.103	1.00	23.93	BBBB
ATOM	1489	N	VAL	176	39.852	43.761	55.011	1.00	24.25	BBBB
ATOM	1490	H	VAL	176	40.832	43.727	54.897	1.00	0.00	BBBB
ATOM	1491	CA	VAL	176	39.259	44.145	56.291	1.00	25.87	BBBB
ATOM	1492	CB	VAL	176	39.486	43.065	57.389	1.00	26.10	BBBB
ATOM	1493	CG1	VAL	176	38.747	43.442	58.658	1.00	23.79	BBBB
ATOM	1494	CG2	VAL	176	39.012	41.709	56.905	1.00	23.00	BBBB
ATOM	1495	C	VAL	176	39.844	45.471	56.765	1.00	26.17	BBBB
ATOM	1496	O	VAL	176	41.061	45.661	56.745	1.00	25.83	BBBB
ATOM	1497	N	ASP	177	38.961	46.423	57.064	1.00	30.10	BBBB
ATOM	1498	H	ASP	177	38.013	46.197	56.991	1.00	0.00	BBBB

ATOM	1499	CA	ASP	177	39.348	47.735	57.593	1.00	31.23 BBBB
ATOM	1500	CB	ASP	177	39.656	47.634	59.088	1.00	37.10 BBBB
ATOM	1501	CG	ASP	177	38.434	47.241	59.924	1.00	43.92 BBBB
ATOM	1502	OD1	ASP	177	38.610	46.901	61.115	1.00	45.60 BBBB
ATOM	1503	OD2	ASP	177	37.299	47.249	59.388	1.00	48.64 BBBB
ATOM	1504	C	ASP	177	40.540	48.339	56.864	1.00	30.72 BBBB
ATOM	1505	O	ASP	177	41.580	48.596	57.472	1.00	31.89 BBBB
ATOM	1506	N	GLY	178	40.456	48.339	55.535	1.00	29.77 BBBB
ATOM	1507	H	GLY	178	39.689	47.869	55.161	1.00	0.00 BBBB
ATOM	1508	CA	GLY	178	41.435	49.035	54.715	1.00	30.17 BBBB
ATOM	1509	C	GLY	178	42.725	48.298	54.407	1.00	28.13 BBBB
ATOM	1510	O	GLY	178	43.607	48.833	53.738	1.00	28.30 BBBB
ATOM	1511	N	HIS	179	42.842	47.061	54.877	1.00	27.85 BBBB
ATOM	1512	H	HIS	179	42.131	46.661	55.410	1.00	0.00 BBBB
ATOM	1513	CA	HIS	179	44.031	46.272	54.588	1.00	27.46 BBBB
ATOM	1514	CB	HIS	179	44.917	46.186	55.822	1.00	31.40 BBBB
ATOM	1515	CG	HIS	179	45.299	47.526	56.364	1.00	39.75 BBBB
ATOM	1516	CD2	HIS	179	46.221	48.424	55.942	1.00	39.28 BBBB
ATOM	1517	ND1	HIS	179	44.541	48.174	57.317	1.00	39.91 BBBB
ATOM	1518	HD1	HIS	179	43.819	47.792	57.859	1.00	0.00 BBBB
ATOM	1519	CE1	HIS	179	44.971	49.417	57.448	1.00	42.46 BBBB
ATOM	1520	NE2	HIS	179	45.990	49.592	56.624	1.00	43.86 BBBB
ATOM	1521	HE2	HIS	179	46.444	50.449	56.500	1.00	0.00 BBBB
ATOM	1522	C	HIS	179	43.726	44.885	54.074	1.00	26.07 BBBB
ATOM	1523	O	HIS	179	42.624	44.362	54.290	1.00	24.18 BBBB
ATOM	1524	N	LEU	180	44.642	44.397	53.238	1.00	23.29 BBBB
ATOM	1525	H	LEU	180	45.398	44.952	52.987	1.00	0.00 BBBB
ATOM	1526	CA	LEU	180	44.582	43.062	52.646	1.00	23.82 BBBB
ATOM	1527	CB	LEU	180	45.308	43.066	51.299	1.00	22.59 BBBB
ATOM	1528	CG	LEU	180	45.434	41.775	50.491	1.00	22.52 BBBB
ATOM	1529	CD1	LEU	180	44.070	41.212	50.147	1.00	22.19 BBBB
ATOM	1530	CD2	LEU	180	46.206	42.094	49.223	1.00	24.25 BBBB
ATOM	1531	C	LEU	180	45.208	42.006	53.560	1.00	22.99 BBBB
ATOM	1532	O	LEU	180	46.402	42.059	53.876	1.00	23.65 BBBB
ATOM	1533	N	TYR	181	44.395	41.055	53.994	1.00	20.53 BBBB
ATOM	1534	H	TYR	181	43.466	41.057	53.679	1.00	0.00 BBBB
ATOM	1535	CA	TYR	181	44.867	40.015	54.896	1.00	18.31 BBBB
ATOM	1536	CB	TYR	181	43.975	39.921	56.143	1.00	16.99 BBBB
ATOM	1537	CG	TYR	181	44.141	41.097	57.092	1.00	19.91 BBBB
ATOM	1538	CD1	TYR	181	43.303	42.210	57.001	1.00	21.59 BBBB
ATOM	1539	CE1	TYR	181	43.493	43.329	57.820	1.00	22.12 BBBB
ATOM	1540	CD2	TYR	181	45.172	41.126	58.034	1.00	19.21 BBBB
ATOM	1541	CE2	TYR	181	45.361	42.236	58.863	1.00	20.75 BBBB

ATOM	1542	CZ	TYR	181	44.515	43.332	58.751	1.00	21.81	BBBB
ATOM	1543	OH	TYR	181	44.643	44.415	59.594	1.00	21.78	BBBB
ATOM	1544	HH	TYR	181	44.032	45.113	59.325	1.00	0.00	BBBB
ATOM	1545	C	TYR	181	44.903	38.688	54.188	1.00	17.40	BBBB
ATOM	1546	O	TYR	181	44.002	38.360	53.412	1.00	16.63	BBBB
ATOM	1547	N	GLU	182	46.057	38.049	54.272	1.00	15.21	BBBB
ATOM	1548	H	GLU	182	46.791	38.531	54.688	1.00	0.00	BBBB
ATOM	1549	CA	GLU	182	46.195	36.674	53.845	1.00	13.51	BBBB
ATOM	1550	CB	GLU	182	47.642	36.373	53.429	1.00	11.85	BBBB
ATOM	1551	CG	GLU	182	47.980	34.891	53.391	1.00	11.30	BBBB
ATOM	1552	CD	GLU	182	49.468	34.613	53.230	1.00	14.98	BBBB
ATOM	1553	OE1	GLU	182	50.230	35.561	52.970	1.00	15.00	BBBB
ATOM	1554	OE2	GLU	182	49.865	33.441	53.342	1.00	14.93	BBBB
ATOM	1555	C	GLU	182	45.847	35.874	55.071	1.00	14.99	BBBB
ATOM	1556	O	GLU	182	46.449	36.065	56.140	1.00	15.11	BBBB
ATOM	1557	N	LEU	183	44.835	35.028	54.934	1.00	12.67	BBBB
ATOM	1558	H	LEU	183	44.349	35.063	54.090	1.00	0.00	BBBB
ATOM	1559	CA	LEU	183	44.445	34.127	56.002	1.00	12.90	BBBB
ATOM	1560	CB	LEU	183	42.942	34.265	56.275	1.00	15.03	BBBB
ATOM	1561	CG	LEU	183	42.456	35.701	56.541	1.00	15.56	BBBB
ATOM	1562	CD1	LEU	183	40.936	35.730	56.613	1.00	15.32	BBBB
ATOM	1563	CD2	LEU	183	43.039	36.234	57.858	1.00	16.89	BBBB
ATOM	1564	C	LEU	183	44.829	32.687	55.682	1.00	11.08	BBBB
ATOM	1565	O	LEU	183	44.329	32.073	54.757	1.00	10.71	BBBB
ATOM	1566	N	ASP	184	45.836	32.203	56.387	1.00	13.16	BBBB
ATOM	1567	H	ASP	184	46.219	32.837	57.037	1.00	0.00	BBBB
ATOM	1568	CA	ASP	184	46.376	30.859	56.192	1.00	14.34	BBBB
ATOM	1569	CB	ASP	184	47.759	30.964	55.527	1.00	14.33	BBBB
ATOM	1570	CG	ASP	184	48.432	29.613	55.334	1.00	15.44	BBBB
ATOM	1571	OD1	ASP	184	49.409	29.556	54.558	1.00	16.13	BBBB
ATOM	1572	OD2	ASP	184	47.999	28.611	55.936	1.00	14.97	BBBB
ATOM	1573	C	ASP	184	46.511	30.220	57.570	1.00	16.33	BBBB
ATOM	1574	O	ASP	184	47.346	30.644	58.356	1.00	16.05	BBBB
ATOM	1575	N	GLY	185	45.728	29.185	57.850	1.00	16.42	BBBB
ATOM	1576	H	GLY	185	45.154	28.820	57.141	1.00	0.00	BBBB
ATOM	1577	CA	GLY	185	45.700	28.623	59.199	1.00	17.67	BBBB
ATOM	1578	C	GLY	185	46.987	27.929	59.618	1.00	18.28	BBBB
ATOM	1579	O	GLY	185	47.130	27.512	60.762	1.00	18.70	BBBB
ATOM	1580	N	ARG	186	47.867	27.685	58.650	1.00	19.25	BBBB
ATOM	1581	H	ARG	186	47.535	27.821	57.748	1.00	0.00	BBBB
ATOM	1582	CA	ARG	186	49.198	27.155	58.937	1.00	19.66	BBBB
ATOM	1583	CB	ARG	186	49.894	26.714	57.644	1.00	17.80	BBBB
ATOM	1584	CG	ARG	186	49.340	25.429	57.048	1.00	21.14	BBBB

ATOM	1585	CD	ARG	186	50.144	24.967	55.843	1.00	24.27	BBBB
ATOM	1586	NE	ARG	186	51.324	24.196	56.228	1.00	28.78	BBBB
ATOM	1587	HE	ARG	186	51.195	23.379	56.760	1.00	0.00	BBBB
ATOM	1588	CZ	ARG	186	52.575	24.524	55.907	1.00	29.73	BBBB
ATOM	1589	NH1	ARG	186	53.569	23.710	56.236	1.00	28.20	BBBB
ATOM	1590	HH11	ARG	186	54.505	23.980	56.016	1.00	0.00	BBBB
ATOM	1591	HH12	ARG	186	53.390	22.890	56.781	1.00	0.00	BBBB
ATOM	1592	NH2	ARG	186	52.836	25.644	55.239	1.00	27.79	BBBB
ATOM	1593	HH21	ARG	186	52.060	26.225	55.039	1.00	0.00	BBBB
ATOM	1594	HH22	ARG	186	53.769	25.882	54.957	1.00	0.00	BBBB
ATOM	1595	C	ARG	186	50.068	28.189	59.644	1.00	18.52	BBBB
ATOM	1596	O	ARG	186	51.114	27.871	60.187	1.00	23.14	BBBB
ATOM	1597	N	LYS	187	49.713	29.450	59.504	1.00	16.47	BBBB
ATOM	1598	H	LYS	187	48.842	29.675	59.124	1.00	0.00	BBBB
ATOM	1599	CA	LYS	187	50.543	30.506	60.036	1.00	17.08	BBBB
ATOM	1600	CB	LYS	187	50.565	31.671	59.049	1.00	13.37	BBBB
ATOM	1601	CG	LYS	187	51.244	31.276	57.737	1.00	11.69	BBBB
ATOM	1602	CD	LYS	187	51.391	32.462	56.792	1.00	12.40	BBBB
ATOM	1603	CE	LYS	187	52.035	32.009	55.491	1.00	12.88	BBBB
ATOM	1604	NZ	LYS	187	52.331	33.162	54.604	1.00	12.97	BBBB
ATOM	1605	HZ1	LYS	187	52.773	33.902	55.169	1.00	0.00	BBBB
ATOM	1606	HZ2	LYS	187	51.461	33.563	54.210	1.00	0.00	BBBB
ATOM	1607	HZ3	LYS	187	52.951	32.823	53.846	1.00	0.00	BBBB
ATOM	1608	C	LYS	187	50.068	30.931	61.426	1.00	20.11	BBBB
ATOM	1609	O	LYS	187	48.968	30.575	61.849	1.00	18.37	BBBB
ATOM	1610	N	PRO	188	50.942	31.591	62.199	1.00	19.32	BBBB
ATOM	1611	CD	PRO	188	52.404	31.644	62.017	1.00	19.27	BBBB
ATOM	1612	CA	PRO	188	50.522	31.951	63.565	1.00	20.87	BBBB
ATOM	1613	CB	PRO	188	51.831	32.398	64.248	1.00	20.84	BBBB
ATOM	1614	CG	PRO	188	52.852	32.527	63.125	1.00	20.92	BBBB
ATOM	1615	C	PRO	188	49.442	33.047	63.618	1.00	18.51	BBBB
ATOM	1616	O	PRO	188	48.838	33.303	64.661	1.00	18.14	BBBB
ATOM	1617	N	PHE	189	49.258	33.744	62.506	1.00	16.00	BBBB
ATOM	1618	H	PHE	189	49.673	33.425	61.681	1.00	0.00	BBBB
ATOM	1619	CA	PHE	189	48.441	34.954	62.474	1.00	14.73	BBBB
ATOM	1620	CB	PHE	189	49.161	36.151	63.126	1.00	19.72	BBBB
ATOM	1621	CG	PHE	189	50.668	36.158	62.952	1.00	18.33	BBBB
ATOM	1622	CD1	PHE	189	51.248	36.237	61.693	1.00	19.90	BBBB
ATOM	1623	CD2	PHE	189	51.502	36.056	64.061	1.00	24.46	BBBB
ATOM	1624	CE1	PHE	189	52.632	36.200	61.537	1.00	18.74	BBBB
ATOM	1625	CE2	PHE	189	52.891	36.026	63.913	1.00	23.39	BBBB
ATOM	1626	CZ	PHE	189	53.449	36.090	62.650	1.00	20.37	BBBB
ATOM	1627	C	PHE	189	48.102	35.292	61.035	1.00	14.51	BBBB

ATOM	1628	O	PHE	189	48.654	34.694	60.113	1.00	14.41	BBBB
ATOM	1629	N	PRO	190	47.090	36.150	60.829	1.00	18.27	BBBB
ATOM	1630	CD	PRO	190	46.101	36.625	61.817	1.00	16.54	BBBB
ATOM	1631	CA	PRO	190	46.885	36.773	59.516	1.00	15.88	BBBB
ATOM	1632	CB	PRO	190	45.744	37.757	59.764	1.00	14.74	BBBB
ATOM	1633	CG	PRO	190	45.020	37.200	60.949	1.00	13.70	BBBB
ATOM	1634	C	PRO	190	48.155	37.495	59.086	1.00	18.93	BBBB
ATOM	1635	O	PRO	190	48.944	37.917	59.937	1.00	18.46	BBBB
ATOM	1636	N	ILE	191	48.385	37.566	57.781	1.00	16.14	BBBB
ATOM	1637	H	ILE	191	47.811	37.011	57.212	1.00	0.00	BBBB
ATOM	1638	CA	ILE	191	49.478	38.380	57.235	1.00	18.56	BBBB
ATOM	1639	CB	ILE	191	50.307	37.590	56.177	1.00	15.52	BBBB
ATOM	1640	CG2	ILE	191	51.435	38.471	55.632	1.00	15.36	BBBB
ATOM	1641	CG1	ILE	191	50.814	36.270	56.771	1.00	14.75	BBBB
ATOM	1642	CD	ILE	191	51.668	36.421	58.031	1.00	14.66	BBBB
ATOM	1643	C	ILE	191	48.922	39.646	56.584	1.00	19.70	BBBB
ATOM	1644	O	ILE	191	48.062	39.578	55.691	1.00	17.72	BBBB
ATOM	1645	N	ASN	192	49.352	40.799	57.091	1.00	19.71	BBBB
ATOM	1646	H	ASN	192	49.955	40.764	57.864	1.00	0.00	BBBB
ATOM	1647	CA	ASN	192	48.901	42.083	56.564	1.00	22.28	BBBB
ATOM	1648	CB	ASN	192	49.056	43.189	57.615	1.00	22.85	BBBB
ATOM	1649	CG	ASN	192	48.416	44.496	57.183	1.00	20.68	BBBB
ATOM	1650	OD1	ASN	192	48.436	44.855	56.012	1.00	26.53	BBBB
ATOM	1651	ND2	ASN	192	47.814	45.191	58.122	1.00	19.89	BBBB
ATOM	1652	HD21	ASN	192	47.456	46.053	57.850	1.00	0.00	BBBB
ATOM	1653	HD22	ASN	192	47.775	44.794	59.012	1.00	0.00	BBBB
ATOM	1654	C	ASN	192	49.725	42.422	55.327	1.00	23.91	BBBB
ATOM	1655	O	ASN	192	50.945	42.513	55.405	1.00	24.94	BBBB
ATOM	1656	N	HIS	193	49.070	42.470	54.169	1.00	20.91	BBBB
ATOM	1657	H	HIS	193	48.105	42.354	54.248	1.00	0.00	BBBB
ATOM	1658	CA	HIS	193	49.767	42.707	52.912	1.00	20.25	BBBB
ATOM	1659	CB	HIS	193	49.263	41.761	51.834	1.00	20.01	BBBB
ATOM	1660	CG	HIS	193	49.789	40.375	51.968	1.00	17.79	BBBB
ATOM	1661	CD2	HIS	193	49.220	39.255	52.470	1.00	16.56	BBBB
ATOM	1662	ND1	HIS	193	51.053	40.007	51.553	1.00	18.33	BBBB
ATOM	1663	HD1	HIS	193	51.743	40.596	51.162	1.00	0.00	BBBB
ATOM	1664	CE1	HIS	193	51.234	38.723	51.776	1.00	20.03	BBBB
ATOM	1665	NE2	HIS	193	50.135	38.237	52.335	1.00	19.35	BBBB
ATOM	1666	HE2	HIS	193	49.908	37.313	52.494	1.00	0.00	BBBB
ATOM	1667	C	HIS	193	49.639	44.132	52.422	1.00	22.80	BBBB
ATOM	1668	O	HIS	193	49.923	44.426	51.253	1.00	29.84	BBBB
ATOM	1669	N	GLY	194	49.166	45.005	53.296	1.00	20.96	BBBB
ATOM	1670	H	GLY	194	48.902	44.693	54.183	1.00	0.00	BBBB

ATOM	1671	CA	GLY	194	49.081	46.400	52.948	1.00	24.33	BBBB
ATOM	1672	C	GLY	194	47.666	46.870	52.713	1.00	26.93	BBBB
ATOM	1673	O	GLY	194	46.698	46.192	53.033	1.00	26.69	BBBB
ATOM	1674	N	GLU	195	47.552	48.092	52.226	1.00	29.16	BBBB
ATOM	1675	H	GLU	195	48.399	48.549	52.034	1.00	0.00	BBBB
ATOM	1676	CA	GLU	195	46.259	48.704	52.028	1.00	33.78	BBBB
ATOM	1677	CB	GLU	195	46.428	50.216	51.875	1.00	39.83	BBBB
ATOM	1678	CG	GLU	195	47.130	50.868	53.070	1.00	50.66	BBBB
ATOM	1679	CD	GLU	195	46.813	52.348	53.202	1.00	57.04	BBBB
ATOM	1680	OE1	GLU	195	46.101	52.716	54.167	1.00	59.34	BBBB
ATOM	1681	OE2	GLU	195	47.273	53.138	52.344	1.00	59.02	BBBB
ATOM	1682	C	GLU	195	45.509	48.121	50.830	1.00	33.75	BBBB
ATOM	1683	O	GLU	195	46.112	47.615	49.876	1.00	32.74	BBBB
ATOM	1684	N	THR	196	44.185	48.102	50.947	1.00	34.10	BBBB
ATOM	1685	H	THR	196	43.806	48.428	51.792	1.00	0.00	BBBB
ATOM	1686	CA	THR	196	43.301	47.694	49.860	1.00	31.68	BBBB
ATOM	1687	CB	THR	196	43.282	46.156	49.687	1.00	29.60	BBBB
ATOM	1688	OG1	THR	196	42.575	45.819	48.494	1.00	29.82	BBBB
ATOM	1689	HG1	THR	196	43.221	45.594	47.824	1.00	0.00	BBBB
ATOM	1690	CG2	THR	196	42.599	45.489	50.864	1.00	28.59	BBBB
ATOM	1691	C	THR	196	41.882	48.182	50.156	1.00	35.53	BBBB
ATOM	1692	O	THR	196	41.587	48.676	51.258	1.00	37.07	BBBB
ATOM	1693	N	SER	197	40.978	47.955	49.210	1.00	34.92	BBBB
ATOM	1694	H	SER	197	41.230	47.386	48.452	1.00	0.00	BBBB
ATOM	1695	CA	SER	197	39.614	48.467	49.318	1.00	35.94	BBBB
ATOM	1696	CB	SER	197	39.469	49.780	48.532	1.00	35.57	BBBB
ATOM	1697	OG	SER	197	39.576	49.545	47.140	1.00	38.66	BBBB
ATOM	1698	HG	SER	197	40.504	49.351	46.898	1.00	0.00	BBBB
ATOM	1699	C	SER	197	38.645	47.453	48.752	1.00	34.85	BBBB
ATOM	1700	O	SER	197	39.049	46.547	48.023	1.00	35.33	BBBB
ATOM	1701	N	ASP	198	37.357	47.688	48.978	1.00	35.50	BBBB
ATOM	1702	H	ASP	198	37.148	48.444	49.563	1.00	0.00	BBBB
ATOM	1703	CA	ASP	198	36.314	46.892	48.341	1.00	34.12	BBBB
ATOM	1704	CB	ASP	198	34.941	47.470	48.669	1.00	38.91	BBBB
ATOM	1705	CG	ASP	198	34.511	47.206	50.096	1.00	42.65	BBBB
ATOM	1706	OD1	ASP	198	35.030	46.260	50.727	1.00	41.80	BBBB
ATOM	1707	OD2	ASP	198	33.596	47.923	50.565	1.00	48.05	BBBB
ATOM	1708	C	ASP	198	36.490	46.878	46.821	1.00	32.19	BBBB
ATOM	1709	O	ASP	198	36.313	45.851	46.159	1.00	30.03	BBBB
ATOM	1710	N	GLU	199	36.900	48.018	46.283	1.00	31.22	BBBB
ATOM	1711	H	GLU	199	37.225	48.737	46.860	1.00	0.00	BBBB
ATOM	1712	CA	GLU	199	36.906	48.216	44.845	1.00	32.54	BBBB
ATOM	1713	CB	GLU	199	36.896	49.714	44.535	1.00	35.99	BBBB

ATOM	1714	CG	GLU	199	36.693	50.048	43.064	0.00	35.94	BBBB
ATOM	1715	CD	GLU	199	36.794	51.537	42.786	0.00	36.71	BBBB
ATOM	1716	OE1	GLU	199	35.736	52.191	42.663	0.00	36.92	BBBB
ATOM	1717	OE2	GLU	199	37.927	52.049	42.678	0.00	36.88	BBBB
ATOM	1718	C	GLU	199	38.102	47.549	44.182	1.00	33.23	BBBB
ATOM	1719	O	GLU	199	38.022	47.094	43.042	1.00	35.95	BBBB
ATOM	1720	N	THR	200	39.211	47.466	44.900	1.00	31.19	BBBB
ATOM	1721	H	THR	200	39.195	47.740	45.842	1.00	0.00	BBBB
ATOM	1722	CA	THR	200	40.448	47.014	44.286	1.00	29.88	BBBB
ATOM	1723	CB	THR	200	41.550	48.032	44.504	1.00	30.21	BBBB
ATOM	1724	OG1	THR	200	41.741	48.226	45.912	1.00	33.62	BBBB
ATOM	1725	HG1	THR	200	42.647	47.966	46.081	1.00	0.00	BBBB
ATOM	1726	CG2	THR	200	41.169	49.349	43.883	1.00	32.46	BBBB
ATOM	1727	C	THR	200	40.929	45.671	44.820	1.00	29.23	BBBB
ATOM	1728	O	THR	200	42.026	45.222	44.478	1.00	29.48	BBBB
ATOM	1729	N	LEU	201	40.112	45.022	45.645	1.00	26.09	BBBB
ATOM	1730	H	LEU	201	39.262	45.438	45.894	1.00	0.00	BBBB
ATOM	1731	CA	LEU	201	40.495	43.741	46.234	1.00	24.40	BBBB
ATOM	1732	CB	LEU	201	39.347	43.148	47.081	1.00	23.41	BBBB
ATOM	1733	CG	LEU	201	39.696	41.793	47.723	1.00	22.38	BBBB
ATOM	1734	CD1	LEU	201	40.856	41.971	48.697	1.00	19.43	BBBB
ATOM	1735	CD2	LEU	201	38.518	41.208	48.436	1.00	20.99	BBBB
ATOM	1736	C	LEU	201	41.004	42.699	45.222	1.00	24.10	BBBB
ATOM	1737	O	LEU	201	42.039	42.067	45.458	1.00	21.46	BBBB
ATOM	1738	N	LEU	202	40.311	42.538	44.092	1.00	23.29	BBBB
ATOM	1739	H	LEU	202	39.506	43.062	43.967	1.00	0.00	BBBB
ATOM	1740	CA	LEU	202	40.734	41.578	43.076	1.00	23.58	BBBB
ATOM	1741	CB	LEU	202	39.769	41.570	41.881	1.00	22.67	BBBB
ATOM	1742	CG	LEU	202	40.123	40.532	40.796	1.00	23.83	BBBB
ATOM	1743	CD1	LEU	202	40.060	39.135	41.378	1.00	23.97	BBBB
ATOM	1744	CD2	LEU	202	39.184	40.629	39.609	1.00	24.35	BBBB
ATOM	1745	C	LEU	202	42.167	41.851	42.583	1.00	24.21	BBBB
ATOM	1746	O	LEU	202	43.008	40.966	42.636	1.00	25.26	BBBB
ATOM	1747	N	GLU	203	42.466	43.102	42.246	1.00	24.33	BBBB
ATOM	1748	H	GLU	203	41.743	43.756	42.344	1.00	0.00	BBBB
ATOM	1749	CA	GLU	203	43.797	43.484	41.769	1.00	26.76	BBBB
ATOM	1750	CB	GLU	203	43.822	44.942	41.347	1.00	30.49	BBBB
ATOM	1751	CG	GLU	203	43.153	45.233	40.052	1.00	44.95	BBBB
ATOM	1752	CD	GLU	203	41.698	44.838	40.070	1.00	53.45	BBBB
ATOM	1753	OE1	GLU	203	40.954	45.332	40.954	1.00	53.33	BBBB
ATOM	1754	OE2	GLU	203	41.329	43.967	39.245	1.00	59.22	BBBB
ATOM	1755	C	GLU	203	44.853	43.314	42.844	1.00	25.56	BBBB
ATOM	1756	O	GLU	203	45.928	42.792	42.585	1.00	26.26	BBBB

ATOM	1757	N	ASP	204	44.567	43.846	44.027	1.00	24.51	BBBB
ATOM	1758	H	ASP	204	43.705	44.302	44.116	1.00	0.00	BBBB
ATOM	1759	CA	ASP	204	45.501	43.796	45.156	1.00	26.31	BBBB
ATOM	1760	CB	ASP	204	44.968	44.665	46.303	1.00	24.27	BBBB
ATOM	1761	CG	ASP	204	44.849	46.143	45.911	1.00	30.80	BBBB
ATOM	1762	OD1	ASP	204	45.337	46.520	44.820	1.00	31.50	BBBB
ATOM	1763	OD2	ASP	204	44.263	46.935	46.682	1.00	30.67	BBBB
ATOM	1764	C	ASP	204	45.807	42.370	45.646	1.00	25.41	BBBB
ATOM	1765	O	ASP	204	46.962	42.039	45.946	1.00	25.28	BBBB
ATOM	1766	N	ALA	205	44.800	41.501	45.617	1.00	21.59	BBBB
ATOM	1767	H	ALA	205	43.910	41.827	45.372	1.00	0.00	BBBB
ATOM	1768	CA	ALA	205	44.993	40.103	45.971	1.00	20.94	BBBB
ATOM	1769	CB	ALA	205	43.635	39.417	46.210	1.00	19.51	BBBB
ATOM	1770	C	ALA	205	45.792	39.335	44.919	1.00	23.38	BBBB
ATOM	1771	O	ALA	205	46.581	38.449	45.245	1.00	23.30	BBBB
ATOM	1772	N	ILE	206	45.561	39.641	43.648	1.00	24.50	BBBB
ATOM	1773	H	ILE	206	44.861	40.303	43.440	1.00	0.00	BBBB
ATOM	1774	CA	ILE	206	46.316	38.996	42.577	1.00	23.48	BBBB
ATOM	1775	CB	ILE	206	45.614	39.219	41.198	1.00	25.93	BBBB
ATOM	1776	CG2	ILE	206	46.548	38.880	40.046	1.00	28.83	BBBB
ATOM	1777	CG1	ILE	206	44.306	38.403	41.140	1.00	24.14	BBBB
ATOM	1778	CD	ILE	206	44.460	36.916	41.168	1.00	22.80	BBBB
ATOM	1779	C	ILE	206	47.794	39.462	42.556	1.00	21.88	BBBB
ATOM	1780	O	ILE	206	48.690	38.647	42.356	1.00	22.55	BBBB
ATOM	1781	N	GLU	207	48.053	40.704	42.952	1.00	23.56	BBBB
ATOM	1782	H	GLU	207	47.305	41.330	43.060	1.00	0.00	BBBB
ATOM	1783	CA	GLU	207	49.433	41.154	43.183	1.00	24.83	BBBB
ATOM	1784	CB	GLU	207	49.462	42.580	43.716	1.00	28.57	BBBB
ATOM	1785	CG	GLU	207	48.898	43.637	42.782	1.00	38.93	BBBB
ATOM	1786	CD	GLU	207	49.151	43.327	41.327	1.00	46.17	BBBB
ATOM	1787	OE1	GLU	207	50.338	43.204	40.949	1.00	46.72	BBBB
ATOM	1788	OE2	GLU	207	48.157	43.174	40.573	1.00	53.27	BBBB
ATOM	1789	C	GLU	207	50.150	40.256	44.176	1.00	26.10	BBBB
ATOM	1790	O	GLU	207	51.250	39.775	43.910	1.00	24.31	BBBB
ATOM	1791	N	VAL	208	49.502	39.990	45.311	1.00	26.00	BBBB
ATOM	1792	H	VAL	208	48.670	40.493	45.482	1.00	0.00	BBBB
ATOM	1793	CA	VAL	208	50.015	39.009	46.273	1.00	24.95	BBBB
ATOM	1794	CB	VAL	208	49.129	38.946	47.549	1.00	24.26	BBBB
ATOM	1795	CG1	VAL	208	49.612	37.844	48.496	1.00	23.70	BBBB
ATOM	1796	CG2	VAL	208	49.155	40.275	48.244	1.00	21.67	BBBB
ATOM	1797	C	VAL	208	50.151	37.597	45.674	1.00	26.78	BBBB
ATOM	1798	O	VAL	208	51.154	36.911	45.916	1.00	29.35	BBBB
ATOM	1799	N	CYS	209	49.183	37.171	44.863	1.00	22.50	BBBB

ATOM	1800	H	CYS	209	48.377	37.730	44.776	1.00	0.00 BBBB
ATOM	1801	CA	CYS	209	49.296	35.871	44.203	1.00	25.82 BBBB
ATOM	1802	CB	CYS	209	48.036	35.546	43.395	1.00	26.60 BBBB
ATOM	1803	SG	CYS	209	46.543	35.387	44.399	1.00	28.29 BBBB
ATOM	1804	C	CYS	209	50.518	35.796	43.277	1.00	26.91 BBBB
ATOM	1805	O	CYS	209	51.212	34.772	43.229	1.00	25.85 BBBB
ATOM	1806	N	LYS	210	50.766	36.875	42.537	1.00	29.99 BBBB
ATOM	1807	H	LYS	210	50.179	37.646	42.629	1.00	0.00 BBBB
ATOM	1808	CA	LYS	210	51.919	36.947	41.631	1.00	34.45 BBBB
ATOM	1809	CB	LYS	210	51.820	38.188	40.737	1.00	33.88 BBBB
ATOM	1810	CG	LYS	210	50.789	38.051	39.625	1.00	39.79 BBBB
ATOM	1811	CD	LYS	210	50.793	39.254	38.687	1.00	40.27 BBBB
ATOM	1812	CE	LYS	210	49.886	40.351	39.183	1.00	37.63 BBBB
ATOM	1813	NZ	LYS	210	50.066	41.602	38.404	1.00	42.21 BBBB
ATOM	1814	HZ1	LYS	210	49.737	41.477	37.425	1.00	0.00 BBBB
ATOM	1815	HZ2	LYS	210	51.073	41.858	38.408	1.00	0.00 BBBB
ATOM	1816	HZ3	LYS	210	49.517	42.363	38.853	1.00	0.00 BBBB
ATOM	1817	C	LYS	210	53.255	36.951	42.395	1.00	35.34 BBBB
ATOM	1818	O	LYS	210	54.226	36.338	41.956	1.00	37.89 BBBB
ATOM	1819	N	LYS	211	53.252	37.513	43.601	1.00	33.64 BBBB
ATOM	1820	H	LYS	211	52.463	38.041	43.867	1.00	0.00 BBBB
ATOM	1821	CA	LYS	211	54.402	37.428	44.485	1.00	33.59 BBBB
ATOM	1822	CB	LYS	211	54.171	38.308	45.710	1.00	37.75 BBBB
ATOM	1823	CG	LYS	211	54.711	39.721	45.563	1.00	43.42 BBBB
ATOM	1824	CD	LYS	211	54.109	40.692	46.592	1.00	46.72 BBBB
ATOM	1825	CE	LYS	211	53.933	40.053	47.968	1.00	51.43 BBBB
ATOM	1826	NZ	LYS	211	53.323	41.004	48.941	1.00	56.76 BBBB
ATOM	1827	HZ1	LYS	211	52.474	41.435	48.539	1.00	0.00 BBBB
ATOM	1828	HZ2	LYS	211	54.006	41.758	49.162	1.00	0.00 BBBB
ATOM	1829	HZ3	LYS	211	53.063	40.507	49.815	1.00	0.00 BBBB
ATOM	1830	C	LYS	211	54.693	35.984	44.917	1.00	34.73 BBBB
ATOM	1831	O	LYS	211	55.852	35.548	44.921	1.00	35.11 BBBB
ATOM	1832	N	PHE	212	53.643	35.231	45.243	1.00	29.19 BBBB
ATOM	1833	H	PHE	212	52.776	35.679	45.325	1.00	0.00 BBBB
ATOM	1834	CA	PHE	212	53.775	33.795	45.490	1.00	26.68 BBBB
ATOM	1835	CB	PHE	212	52.394	33.176	45.751	1.00	25.71 BBBB
ATOM	1836	CG	PHE	212	51.936	33.288	47.180	1.00	21.00 BBBB
ATOM	1837	CD1	PHE	212	51.766	34.545	47.774	1.00	19.39 BBBB
ATOM	1838	CD2	PHE	212	51.759	32.137	47.950	1.00	21.10 BBBB
ATOM	1839	CE1	PHE	212	51.434	34.655	49.131	1.00	20.27 BBBB
ATOM	1840	CE2	PHE	212	51.426	32.226	49.303	1.00	21.64 BBBB
ATOM	1841	CZ	PHE	212	51.266	33.497	49.896	1.00	19.69 BBBB
ATOM	1842	C	PHE	212	54.438	33.069	44.308	1.00	27.94 BBBB

ATOM	1843	O	PHE	212	55.379	32.289	44.469	1.00	29.82	BBBB
ATOM	1844	N	MET	213	53.942	33.347	43.114	1.00	28.49	BBBB
ATOM	1845	H	MET	213	53.195	33.982	43.073	1.00	0.00	BBBB
ATOM	1846	CA	MET	213	54.439	32.709	41.909	1.00	31.13	BBBB
ATOM	1847	CB	MET	213	53.534	33.080	40.748	1.00	31.76	BBBB
ATOM	1848	CG	MET	213	52.139	32.540	40.911	1.00	32.92	BBBB
ATOM	1849	SD	MET	213	51.144	32.918	39.495	1.00	39.38	BBBB
ATOM	1850	CE	MET	213	51.471	31.457	38.429	1.00	35.56	BBBB
ATOM	1851	C	MET	213	55.888	33.089	41.596	1.00	32.08	BBBB
ATOM	1852	O	MET	213	56.729	32.220	41.400	1.00	30.06	BBBB
ATOM	1853	N	GLU	214	56.192	34.380	41.688	1.00	36.22	BBBB
ATOM	1854	H	GLU	214	55.459	34.995	41.879	1.00	0.00	BBBB
ATOM	1855	CA	GLU	214	57.558	34.879	41.512	1.00	42.62	BBBB
ATOM	1856	CB	GLU	214	57.613	36.380	41.817	1.00	45.96	BBBB
ATOM	1857	CG	GLU	214	57.738	37.265	40.579	1.00	54.19	BBBB
ATOM	1858	CD	GLU	214	56.753	38.427	40.570	1.00	59.49	BBBB
ATOM	1859	OE1	GLU	214	56.618	39.120	41.609	1.00	62.81	BBBB
ATOM	1860	OE2	GLU	214	56.119	38.648	39.513	1.00	61.93	BBBB
ATOM	1861	C	GLU	214	58.572	34.144	42.392	1.00	44.13	BBBB
ATOM	1862	O	GLU	214	59.653	33.783	41.938	1.00	46.23	BBBB
ATOM	1863	N	ARG	215	58.188	33.867	43.633	1.00	44.81	BBBB
ATOM	1864	H	ARG	215	57.295	34.167	43.910	1.00	0.00	BBBB
ATOM	1865	CA	ARG	215	59.052	33.164	44.573	1.00	47.12	BBBB
ATOM	1866	CB	ARG	215	58.419	33.174	45.959	1.00	49.23	BBBB
ATOM	1867	CG	ARG	215	58.442	34.512	46.634	1.00	54.62	BBBB
ATOM	1868	CD	ARG	215	57.749	34.429	47.970	1.00	59.28	BBBB
ATOM	1869	NE	ARG	215	57.032	35.662	48.269	1.00	63.64	BBBB
ATOM	1870	HE	ARG	215	57.427	36.515	47.986	1.00	0.00	BBBB
ATOM	1871	CZ	ARG	215	55.866	35.700	48.902	1.00	66.38	BBBB
ATOM	1872	NH1	ARG	215	55.281	36.866	49.147	1.00	69.59	BBBB
ATOM	1873	HH11	ARG	215	55.682	37.708	48.788	1.00	0.00	BBBB
ATOM	1874	HH12	ARG	215	54.403	36.895	49.629	1.00	0.00	BBBB
ATOM	1875	NH2	ARG	215	55.298	34.569	49.314	1.00	66.82	BBBB
ATOM	1876	HH21	ARG	215	54.426	34.601	49.803	1.00	0.00	BBBB
ATOM	1877	HH22	ARG	215	55.711	33.685	49.101	1.00	0.00	BBBB
ATOM	1878	C	ARG	215	59.321	31.719	44.166	1.00	48.10	BBBB
ATOM	1879	O	ARG	215	60.245	31.077	44.670	1.00	49.48	BBBB
ATOM	1880	N	ASP	216	58.422	31.164	43.369	1.00	47.98	BBBB
ATOM	1881	H	ASP	216	57.679	31.721	43.049	1.00	0.00	BBBB
ATOM	1882	CA	ASP	216	58.530	29.771	42.972	1.00	47.13	BBBB
ATOM	1883	CB	ASP	216	57.473	28.944	43.711	1.00	47.78	BBBB
ATOM	1884	CG	ASP	216	57.963	27.553	44.093	1.00	50.19	BBBB
ATOM	1885	OD1	ASP	216	58.843	26.996	43.395	1.00	51.39	BBBB

ATOM	1886	OD2	ASP	216	57.431	26.985	45.072	1.00	48.92 BBBB
ATOM	1887	C	ASP	216	58.355	29.651	41.458	1.00	47.63 BBBB
ATOM	1888	O	ASP	216	57.382	29.067	40.979	1.00	48.75 BBBB
ATOM	1889	N	PRO	217	59.337	30.147	40.685	1.00	47.00 BBBB
ATOM	1890	CD	PRO	217	60.664	30.580	41.150	1.00	46.27 BBBB
ATOM	1891	CA	PRO	217	59.102	30.558	39.299	1.00	46.24 BBBB
ATOM	1892	CB	PRO	217	60.344	31.373	38.953	1.00	45.55 BBBB
ATOM	1893	CG	PRO	217	60.942	31.740	40.263	1.00	45.13 BBBB
ATOM	1894	C	PRO	217	58.910	29.393	38.335	1.00	47.66 BBBB
ATOM	1895	O	PRO	217	58.407	29.573	37.230	1.00	50.76 BBBB
ATOM	1896	N	ASP	218	59.325	28.200	38.742	1.00	48.45 BBBB
ATOM	1897	H	ASP	218	59.857	28.156	39.563	1.00	0.00 BBBB
ATOM	1898	CA	ASP	218	59.133	27.016	37.912	1.00	53.00 BBBB
ATOM	1899	CB	ASP	218	60.313	26.054	38.073	1.00	59.27 BBBB
ATOM	1900	CG	ASP	218	61.401	26.286	37.038	1.00	62.24 BBBB
ATOM	1901	OD1	ASP	218	62.264	27.163	37.278	1.00	61.84 BBBB
ATOM	1902	OD2	ASP	218	61.384	25.592	35.990	1.00	64.66 BBBB
ATOM	1903	C	ASP	218	57.847	26.266	38.214	1.00	54.01 BBBB
ATOM	1904	O	ASP	218	57.470	25.352	37.476	1.00	55.95 BBBB
ATOM	1905	N	GLU	219	57.221	26.606	39.339	1.00	54.49 BBBB
ATOM	1906	H	GLU	219	57.552	27.392	39.808	1.00	0.00 BBBB
ATOM	1907	CA	GLU	219	55.990	25.951	39.789	1.00	53.25 BBBB
ATOM	1908	CB	GLU	219	55.744	26.201	41.285	1.00	52.75 BBBB
ATOM	1909	CG	GLU	219	56.022	25.007	42.175	1.00	53.61 BBBB
ATOM	1910	CD	GLU	219	55.203	23.766	41.812	1.00	58.48 BBBB
ATOM	1911	OE1	GLU	219	54.269	23.854	40.975	1.00	58.85 BBBB
ATOM	1912	OE2	GLU	219	55.486	22.689	42.389	1.00	59.45 BBBB
ATOM	1913	C	GLU	219	54.766	26.393	39.001	1.00	51.04 BBBB
ATOM	1914	O	GLU	219	54.415	27.573	38.987	1.00	49.15 BBBB
ATOM	1915	N	LEU	220	54.077	25.419	38.421	1.00	50.75 BBBB
ATOM	1916	H	LEU	220	54.442	24.515	38.500	1.00	0.00 BBBB
ATOM	1917	CA	LEU	220	52.915	25.686	37.578	1.00	51.68 BBBB
ATOM	1918	CB	LEU	220	52.817	24.622	36.484	1.00	54.60 BBBB
ATOM	1919	CG	LEU	220	53.302	24.997	35.084	1.00	58.03 BBBB
ATOM	1920	CD1	LEU	220	52.102	25.048	34.146	1.00	59.62 BBBB
ATOM	1921	CD2	LEU	220	54.038	26.341	35.107	1.00	60.10 BBBB
ATOM	1922	C	LEU	220	51.600	25.726	38.357	1.00	49.77 BBBB
ATOM	1923	O	LEU	220	50.638	26.363	37.929	1.00	48.89 BBBB
ATOM	1924	N	ARG	221	51.605	25.116	39.540	1.00	47.79 BBBB
ATOM	1925	H	ARG	221	52.470	25.007	39.968	1.00	0.00 BBBB
ATOM	1926	CA	ARG	221	50.377	24.679	40.217	1.00	45.16 BBBB
ATOM	1927	CB	ARG	221	50.680	23.414	41.049	1.00	48.94 BBBB
ATOM	1928	CG	ARG	221	51.461	22.326	40.282	1.00	51.97 BBBB

ATOM	1929	CD	ARG	221	52.261	21.403	41.202	1.00	59.14	BBBB
ATOM	1930	NE	ARG	221	51.422	20.383	41.840	1.00	71.38	BBBB
ATOM	1931	HE	ARG	221	50.867	20.658	42.597	1.00	20.00	BBBB
ATOM	1932	CZ	ARG	221	51.331	19.109	41.447	1.00	75.94	BBBB
ATOM	1933	NH1	ARG	221	50.486	18.279	42.061	1.00	75.00	BBBB
ATOM	1934	HH11	ARG	221	49.905	18.611	42.804	1.00	0.00	BBBB
ATOM	1935	HH12	ARG	221	50.417	17.331	41.752	1.00	0.00	BBBB
ATOM	1936	NH2	ARG	221	52.093	18.651	40.453	1.00	77.87	BBBB
ATOM	1937	HH21	ARG	221	52.745	19.258	39.998	1.00	0.00	BBBB
ATOM	1938	HH22	ARG	221	52.024	17.696	40.164	1.00	0.00	BBBB
ATOM	1939	C	ARG	221	49.715	25.774	41.085	1.00	38.76	BBBB
ATOM	1940	O	ARG	221	49.451	25.580	42.271	1.00	33.96	BBBB
ATOM	1941	N	PHE	222	49.467	26.925	40.469	1.00	32.85	BBBB
ATOM	1942	H	PHE	222	49.676	27.003	39.511	1.00	0.00	BBBB
ATOM	1943	CA	PHE	222	48.830	28.062	41.123	1.00	31.95	BBBB
ATOM	1944	CB	PHE	222	49.698	29.321	40.980	1.00	35.29	BBBB
ATOM	1945	CG	PHE	222	50.951	29.306	41.830	1.00	40.24	BBBB
ATOM	1946	CD1	PHE	222	52.100	28.634	41.394	1.00	39.81	BBBB
ATOM	1947	CD2	PHE	222	50.958	29.904	43.095	1.00	38.34	BBBB
ATOM	1948	CE1	PHE	222	53.234	28.540	42.212	1.00	42.53	BBBB
ATOM	1949	CE2	PHE	222	52.087	29.818	43.927	1.00	40.72	BBBB
ATOM	1950	CZ	PHE	222	53.229	29.131	43.485	1.00	41.46	BBBB
ATOM	1951	C	PHE	222	47.485	28.312	40.440	1.00	31.88	BBBB
ATOM	1952	O	PHE	222	47.438	28.497	39.233	1.00	30.41	BBBB
ATOM	1953	N	ASN	223	46.393	28.244	41.195	1.00	25.46	BBBB
ATOM	1954	H	ASN	223	46.518	28.097	42.159	1.00	0.00	BBBB
ATOM	1955	CA	ASN	223	45.057	28.452	40.632	1.00	22.95	BBBB
ATOM	1956	CB	ASN	223	44.389	27.109	40.377	1.00	22.96	BBBB
ATOM	1957	CG	ASN	223	45.190	26.234	39.440	1.00	30.10	BBBB
ATOM	1958	OD1	ASN	223	44.896	26.142	38.249	1.00	39.00	BBBB
ATOM	1959	ND2	ASN	223	46.227	25.602	39.965	1.00	34.21	BBBB
ATOM	1960	HD21	ASN	223	46.685	24.977	39.365	1.00	0.00	BBBB
ATOM	1961	HD22	ASN	223	46.478	25.799	40.881	1.00	0.00	BBBB
ATOM	1962	C	ASN	223	44.211	29.248	41.608	1.00	20.90	BBBB
ATOM	1963	O	ASN	223	44.150	28.908	42.784	1.00	24.24	BBBB
ATOM	1964	N	ALA	224	43.598	30.324	41.148	1.00	19.58	BBBB
ATOM	1965	H	ALA	224	43.709	30.554	40.197	1.00	0.00	BBBB
ATOM	1966	CA	ALA	224	42.785	31.162	42.030	1.00	20.81	BBBB
ATOM	1967	CB	ALA	224	43.349	32.588	42.086	1.00	17.03	BBBB
ATOM	1968	C	ALA	224	41.292	31.199	41.651	1.00	22.36	BBBB
ATOM	1969	O	ALA	224	40.917	30.985	40.490	1.00	23.63	BBBB
ATOM	1970	N	ILE	225	40.443	31.332	42.667	1.00	19.57	BBBB
ATOM	1971	H	ILE	225	40.814	31.262	43.573	1.00	0.00	BBBB

ATOM	1972	CA	ILE	225	39.022	31.595	42.456	1.00	18.02	BBBB
ATOM	1973	CB	ILE	225	38.139	30.383	42.880	1.00	17.65	BBBB
ATOM	1974	CG2	ILE	225	38.420	29.200	41.962	1.00	18.13	BBBB
ATOM	1975	CG1	ILE	225	38.371	30.016	44.361	1.00	16.24	BBBB
ATOM	1976	CD	ILE	225	37.619	28.785	44.847	1.00	14.44	BBBB
ATOM	1977	C	ILE	225	38.605	32.846	43.220	1.00	19.24	BBBB
ATOM	1978	O	ILE	225	39.239	33.210	44.219	1.00	20.04	BBBB
ATOM	1979	N	ALA	226	37.665	33.593	42.648	1.00	16.66	BBBB
ATOM	1980	H	ALA	226	37.258	33.255	41.826	1.00	0.00	BBBB
ATOM	1981	CA	ALA	226	37.224	34.869	43.209	1.00	15.95	BBBB
ATOM	1982	CB	ALA	226	37.347	35.958	42.154	1.00	12.73	BBBB
ATOM	1983	C	ALA	226	35.775	34.757	43.679	1.00	18.07	BBBB
ATOM	1984	O	ALA	226	34.971	34.086	43.019	1.00	18.08	BBBB
ATOM	1985	N	LEU	227	35.492	35.237	44.894	1.00	18.46	BBBB
ATOM	1986	H	LEU	227	36.235	35.501	45.449	1.00	0.00	BBBB
ATOM	1987	CA	LEU	227	34.105	35.292	45.382	1.00	18.63	BBBB
ATOM	1988	CB	LEU	227	34.034	35.223	46.910	1.00	17.78	BBBB
ATOM	1989	CG	LEU	227	32.630	35.026	47.507	1.00	15.56	BBBB
ATOM	1990	CD1	LEU	227	31.934	33.818	46.849	1.00	11.24	BBBB
ATOM	1991	CD2	LEU	227	32.728	34.824	49.026	1.00	16.41	BBBB
ATOM	1992	C	LEU	227	33.421	36.564	44.889	1.00	18.62	BBBB
ATOM	1993	O	LEU	227	33.644	37.662	45.423	1.00	22.19	BBBB
ATOM	1994	N	SER	228	32.617	36.409	43.841	1.00	21.18	BBBB
ATOM	1995	H	SER	228	32.389	35.506	43.551	1.00	0.00	BBBB
ATOM	1996	CA	SER	228	32.101	37.547	43.082	1.00	23.05	BBBB
ATOM	1997	CB	SER	228	32.340	37.337	41.583	1.00	21.38	BBBB
ATOM	1998	OG	SER	228	33.717	37.354	41.256	1.00	27.97	BBBB
ATOM	1999	HG	SER	228	33.938	38.255	40.993	1.00	0.00	BBBB
ATOM	2000	C	SER	228	30.611	37.740	43.319	1.00	22.96	BBBB
ATOM	2001	O	SER	228	29.879	36.774	43.537	1.00	22.70	BBBB
ATOM	2002	N	ALA	229	30.175	38.994	43.240	1.00	25.88	BBBB
ATOM	2003	H	ALA	229	30.865	39.687	43.183	1.00	0.00	BBBB
ATOM	2004	CA	ALA	229	28.755	39.339	43.272	1.00	27.79	BBBB
ATOM	2005	CB	ALA	229	28.576	40.816	43.037	1.00	26.67	BBBB
ATOM	2006	C	ALA	229	27.947	38.557	42.253	1.00	30.41	BBBB
ATOM	2007	O	ALA	229	28.343	38.421	41.091	1.00	28.94	BBBB
ATOM	2008	N	ALA	230	26.875	37.943	42.727	1.00	34.26	BBBB
ATOM	2009	H	ALA	230	26.619	38.098	43.649	1.00	0.00	BBBB
ATOM	2010	CA	ALA	230	25.932	37.264	41.850	1.00	39.79	BBBB
ATOM	2011	CB	ALA	230	26.227	35.769	41.806	1.00	36.23	BBBB
ATOM	2012	C	ALA	230	24.515	37.521	42.353	1.00	43.03	BBBB
ATOM	2013	OT1	ALA	230	24.317	38.529	43.082	1.00	45.31	BBBB
ATOM	2014	OT2	ALA	230	23.610	36.739	41.986	1.00	51.60	BBBB

ATOM 2015 OH2 WAT W 1 51.481 27.762 54.626 1.00 18.55 CCCC
ATOM 2016 H1 WAT W 1 50.686 28.045 55.047 1.00 0.00 CCCC
ATOM 2017 H2 WAT W 1 51.852 28.563 54.263 1.00 0.00 CCCC
ATOM 2018 OH2 WAT W 2 39.370 21.410 52.578 1.00 15.77 CCCC
ATOM 2019 H1 WAT W 2 39.781 20.538 52.650 1.00 0.00 CCCC
ATOM 2020 H2 WAT W 2 38.930 21.344 51.730 1.00 0.00 CCCC
ATOM 2021 OH2 WAT W 3 35.144 43.822 54.730 1.00 48.43 CCCC
ATOM 2022 H1 WAT W 3 35.076 42.857 54.802 1.00 0.00 CCCC
ATOM 2023 H2 WAT W 3 36.016 43.867 54.331 1.00 0.00 CCCC
ATOM 2024 OH2 WAT W 4 39.532 35.665 68.967 1.00 39.36 CCCC
ATOM 2025 H1 WAT W 4 38.850 35.846 69.610 1.00 0.00 CCCC
ATOM 2026 H2 WAT W 4 39.154 34.910 68.494 1.00 0.00 CCCC
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ATOM 2028 H1 WAT W 5 52.330 35.766 53.277 1.00 0.00 CCCC
ATOM 2029 H2 WAT W 5 53.715 35.839 52.604 1.00 0.00 CCCC
ATOM 2030 OH2 WAT W 6 40.635 36.308 64.686 1.00 24.09 CCCC
ATOM 2031 H1 WAT W 6 40.363 37.194 64.430 1.00 0.00 CCCC
ATOM 2032 H2 WAT W 6 40.869 36.450 65.613 1.00 0.00 CCCC
ATOM 2033 OH2 WAT W 7 44.431 27.177 56.600 1.00 19.80 CCCC
ATOM 2034 H1 WAT W 7 44.407 26.205 56.639 1.00 0.00 CCCC
ATOM 2035 H2 WAT W 7 43.481 27.377 56.604 1.00 0.00 CCCC

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ATOM 2062 H2 WAT W 16 36.741 34.794 34.493 1.00 0.00
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ATOM 2063 OH2 WAT W 17 55.000 30.105 39.281 1.00 40.98
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ATOM 2076 H1 WAT W 21 51.070 31.770 53.133 1.00 0.00
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ATOM 2077 H2 WAT W 21 49.874 31.595 52.195 1.00 0.00
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ATOM	2078	OH2	WAT	W	22	47.828	33.973	57.688	1.00	12.70
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ATOM	2079	H1	WAT	W	22	48.249	33.962	58.551	1.00	0.00
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ATOM	2080	H2	WAT	W	22	48.064	33.104	57.362	1.00	0.00
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ATOM	2081	OH2	WAT	W	23	42.063	39.807	64.625	1.00	30.20
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ATOM	2082	H1	WAT	W	23	41.289	39.678	64.069	1.00	0.00
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ATOM	2083	H2	WAT	W	23	42.289	40.734	64.486	1.00	0.00
	CCCC									
ATOM	2084	OH2	WAT	W	24	33.765	20.863	55.211	1.00	29.13
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ATOM	2085	H1	WAT	W	24	33.127	20.926	55.939	1.00	0.00
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ATOM	2086	H2	WAT	W	24	33.724	19.909	55.040	1.00	0.00
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ATOM	2087	OH2	WAT	W	25	36.791	41.478	51.569	1.00	21.42
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ATOM	2088	H1	WAT	W	25	36.666	42.072	52.329	1.00	0.00
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ATOM	2089	H2	WAT	W	25	36.015	40.915	51.732	1.00	0.00
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ATOM	2090	OH2	WAT	W	26	22.888	33.170	41.709	1.00	36.61
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ATOM	2091	H1	WAT	W	26	22.945	34.011	41.225	1.00	0.00
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ATOM	2092	H2	WAT	W	26	23.300	33.483	42.535	1.00	0.00
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ATOM	2093	OH2	WAT	W	27	55.171	39.105	53.741	1.00	42.20
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ATOM	2094	H1	WAT	W	27	55.663	39.042	54.569	1.00	0.00
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ATOM	2095	H2	WAT	W	27	54.806	38.247	53.609	1.00	0.00
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ATOM	2096	OH2	WAT	W	28	28.321	27.402	40.210	1.00	30.51
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ATOM	2097	H1	WAT	W	28	28.268	26.785	39.486	1.00	0.00
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ATOM	2098	H2	WAT	W	28	27.404	27.641	40.409	1.00	0.00
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ATOM 2101 H2 WAT W 29 40.593 16.863 48.822 1.00 0.00
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ATOM 2102 OH2 WAT W 30 39.338 35.289 34.944 1.00 21.05
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ATOM 2103 H1 WAT W 30 38.678 35.160 35.647 1.00 0.00
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ATOM 2105 OH2 WAT W 31 33.019 43.572 48.633 1.00 37.54
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ATOM 2109 H1 WAT W 32 46.890 20.151 52.764 1.00 0.00
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ATOM 2110 H2 WAT W 32 47.134 20.719 51.380 1.00 0.00
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ATOM 2111 OH2 WAT W 33 35.901 43.755 50.596 1.00 26.42
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ATOM 2131 H2 WAT W 39 52.166 41.650 58.142 1.00 0.00
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ATOM 2132 OH2 WAT W 40 22.703 25.095 56.301 1.00 26.37
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ATOM 2137 H2 WAT W 41 30.536 30.996 38.213 1.00 0.00
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ATOM 2139 H1 WAT W 42 26.179 32.491 48.826 1.00 0.00
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ATOM 2140 H2 WAT W 42 26.887 32.882 47.561 1.00 0.00
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ATOM	2141	OH2	WAT	W	43	31.741	35.155	63.468	1.00	34.61
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ATOM	2143	H2	WAT	W	43	32.162	34.352	63.777	1.00	0.00
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ATOM	2144	OH2	WAT	W	44	29.727	30.243	71.731	1.00	31.51
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ATOM	2145	H1	WAT	W	44	28.793	30.072	71.838	1.00	0.00
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ATOM	2146	H2	WAT	W	44	29.847	30.411	70.803	1.00	0.00
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ATOM	2147	OH2	WAT	W	45	36.050	33.785	74.656	1.00	35.20
	CCCC									
ATOM	2148	H1	WAT	W	45	36.659	33.064	74.675	1.00	0.00
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ATOM	2149	H2	WAT	W	45	36.197	34.091	73.746	1.00	0.00
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ATOM	2150	OH2	WAT	W	46	39.267	26.175	75.132	1.00	43.10
	CCCC									
ATOM	2151	H1	WAT	W	46	38.838	26.792	74.559	1.00	0.00
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ATOM	2152	H2	WAT	W	46	38.723	26.196	75.918	1.00	0.00
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ATOM	2153	OH2	WAT	W	47	54.676	27.697	46.296	1.00	31.80
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ATOM	2154	H1	WAT	W	47	54.334	28.563	46.038	1.00	0.00
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ATOM	2155	H2	WAT	W	47	55.478	27.593	45.804	1.00	0.00
	CCCC									
ATOM	2156	OH2	WAT	W	48	43.162	29.556	72.942	1.00	30.66
	CCCC									
ATOM	2157	H1	WAT	W	48	42.388	29.317	73.466	1.00	0.00
	CCCC									
ATOM	2158	H2	WAT	W	48	43.883	29.297	73.531	1.00	0.00
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ATOM	2159	OH2	WAT	W	49	21.891	36.820	56.313	1.00	47.38
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ATOM	2160	H1	WAT	W	49	22.570	37.512	56.485	1.00	0.00
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ATOM	2161	H2	WAT	W	49	21.504	36.758	57.185	1.00	0.00
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ATOM 2163 H1 WAT W 50 22.579 28.751 61.504 1.00 0.00 CCCC
ATOM 2164 H2 WAT W 50 21.888 29.397 62.696 1.00 0.00 CCCC
ATOM 2165 OH2 WAT W 51 24.553 38.700 56.934 1.00 33.19 CCCC
ATOM 2166 H1 WAT W 51 25.457 38.781 57.260 1.00 0.00 CCCC
ATOM 2167 H2 WAT W 51 24.725 38.492 56.006 1.00 0.00 CCCC
ATOM 2168 OH2 WAT W 52 37.071 21.855 45.561 1.00 31.91 CCCC
ATOM 2169 H1 WAT W 52 37.534 21.483 46.320 1.00 0.00 CCCC
ATOM 2170 H2 WAT W 52 36.195 21.465 45.599 1.00 0.00 CCCC
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ATOM 2172 H1 WAT W 53 36.279 41.345 55.006 1.00 0.00 CCCC
ATOM 2173 H2 WAT W 53 35.608 40.534 53.971 1.00 0.00 CCCC
ATOM 2174 OH2 WAT W 54 38.081 43.889 43.261 1.00 36.97 CCCC
ATOM 2175 H1 WAT W 54 37.336 43.702 43.845 1.00 0.00 CCCC
ATOM 2176 H2 WAT W 54 37.896 44.806 43.010 1.00 0.00 CCCC
ATOM 2177 OH2 WAT W 55 35.518 44.375 43.828 1.00 32.62 CCCC
ATOM 2178 H1 WAT W 55 35.620 44.696 44.737 1.00 0.00 CCCC
ATOM 2179 H2 WAT W 55 35.338 45.220 43.378 1.00 0.00 CCCC
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ATOM 2181 H1 WAT W 56 35.701 49.253 50.861 1.00 0.00 CCCC
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ATOM 2203 H2 WAT W 63 26.353 30.264 47.682 1.00 0.00
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ATOM 2204 OH2 WAT W 64 34.733 30.790 35.948 1.00 30.50 CCCC
ATOM 2205 H1 WAT W 64 35.036 29.917 36.213 1.00 0.00 CCCC
ATOM 2206 H2 WAT W 64 34.052 31.003 36.592 1.00 0.00 CCCC
ATOM 2207 OH2 WAT W 65 35.632 43.962 48.122 1.00 39.10 CCCC
ATOM 2208 H1 WAT W 65 35.906 44.347 47.268 1.00 0.00 CCCC
ATOM 2209 H2 WAT W 65 35.638 43.021 47.836 1.00 0.00 CCCC
ATOM 2210 OH2 WAT W 66 41.405 29.179 35.736 1.00 55.14 CCCC
ATOM 2211 H1 WAT W 66 40.685 28.791 36.221 1.00 0.00 CCCC
ATOM 2212 H2 WAT W 66 41.628 29.970 36.248 1.00 0.00 CCCC
ATOM 2213 OH2 WAT W 67 47.648 45.349 48.932 1.00 33.82 CCCC
ATOM 2214 H1 WAT W 67 47.167 46.150 49.188 1.00 0.00 CCCC
ATOM 2215 H2 WAT W 67 47.436 45.253 47.995 1.00 0.00 CCCC
ATOM 2216 OH2 WAT W 68 44.618 17.410 45.691 1.00 53.27 CCCC
ATOM 2217 H1 WAT W 68 43.969 16.949 45.154 1.00 0.00 CCCC
ATOM 2218 H2 WAT W 68 44.364 18.336 45.569 1.00 0.00 CCCC
ATOM 2219 OH2 WAT W 69 27.329 25.288 41.675 1.00 38.34 CCCC
ATOM 2220 H1 WAT W 69 27.449 25.214 40.731 1.00 0.00 CCCC
ATOM 2221 H2 WAT W 69 28.180 25.002 42.032 1.00 0.00 CCCC
ATOM 2222 OH2 WAT W 70 27.444 23.990 68.137 1.00 30.31 CCCC
ATOM 2223 H1 WAT W 70 27.833 24.141 69.000 1.00 0.00 CCCC
ATOM 2224 H2 WAT W 70 26.670 23.444 68.324 1.00 0.00 CCCC

ATOM	2225	OH2	WAT	W	71	48.599	44.086	46.923	1.00	27.72
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	CCCC									
ATOM	2227	H2	WAT	W	71	48.172	43.287	47.257	1.00	0.00
	CCCC									
ATOM	2228	OH2	WAT	W	72	36.454	19.649	44.328	1.00	44.87
	CCCC									
ATOM	2229	H1	WAT	W	72	37.251	19.129	44.245	1.00	0.00
	CCCC									
ATOM	2230	H2	WAT	W	72	36.454	20.184	43.535	1.00	0.00
	CCCC									
ATOM	2231	OH2	WAT	W	73	32.924	36.639	61.406	1.00	49.32
	CCCC									
ATOM	2232	H1	WAT	W	73	32.799	36.561	60.455	1.00	0.00
	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									
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	CCCC									

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CCCC
ATOM 2257 H2 WAT W 81 31.289 16.065 70.482 1.00 0.00
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ATOM 2266 H2 WAT W 84 55.427 30.802 46.067 1.00 0.00
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
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CCCC										
ATOM	2284	H2	WAT	W	90	47.379	25.426	42.760	1.00	0.00
CCCC										
ATOM	2285	OH2	WAT	W	91	44.324	30.773	38.366	1.00	57.11
CCCC										
ATOM	2286	H1	WAT	W	91	44.569	30.042	37.778	1.00	0.00
CCCC										
ATOM	2287	H2	WAT	W	91	43.570	31.159	37.888	1.00	0.00
CCCC										

ATOM 2288 OH2 WAT W 92 49.852 49.467 51.199 1.00 42.65 CCCC
ATOM 2289 H1 WAT W 92 49.178 50.143 51.105 1.00 0.00 CCCC
ATOM 2290 H2 WAT W 92 49.580 48.789 50.564 1.00 0.00 CCCC
ATOM 2291 OH2 WAT W 93 34.519 38.964 70.014 1.00 48.33 CCCC
ATOM 2292 H1 WAT W 93 34.947 39.776 69.730 1.00 0.00 CCCC
ATOM 2293 H2 WAT W 93 34.689 38.364 69.262 1.00 0.00 CCCC
ATOM 2294 OH2 WAT W 94 42.967 50.912 67.215 1.00 39.91 CCCC
ATOM 2295 H1 WAT W 94 43.243 51.292 66.379 1.00 0.00 CCCC
ATOM 2296 H2 WAT W 94 43.165 51.598 67.856 1.00 0.00 CCCC
ATOM 2297 OH2 WAT W 95 47.936 18.190 48.416 1.00 51.10 CCCC
ATOM 2298 H1 WAT W 95 47.199 18.806 48.599 1.00 0.00 CCCC
ATOM 2299 H2 WAT W 95 47.397 17.492 47.973 1.00 0.00 CCCC
ATOM 2300 OH2 WAT W 96 54.886 28.124 62.758 1.00 43.49 CCCC
ATOM 2301 H1 WAT W 96 55.089 28.298 63.676 1.00 0.00 CCCC
ATOM 2302 H2 WAT W 96 55.256 28.857 62.269 1.00 0.00 CCCC
ATOM 2303 OH2 WAT W 97 29.053 34.319 38.220 1.00 39.58 CCCC
ATOM 2304 H1 WAT W 97 28.737 33.944 37.383 1.00 0.00 CCCC
ATOM 2305 H2 WAT W 97 29.432 33.555 38.594 1.00 0.00 CCCC
ATOM 2306 OH2 WAT W 98 29.173 36.014 40.224 1.00 41.00 CCCC
ATOM 2307 H1 WAT W 98 28.640 36.738 39.874 1.00 0.00 CCCC
ATOM 2308 H2 WAT W 98 29.292 35.366 39.527 1.00 0.00 CCCC

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      CCCC
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      CCCC
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      CCCC
ATOM  2312  OH2 WAT  W   100     20.643  35.624  58.943  1.00 43.37
      CCCC
ATOM  2313  H1  WAT  W   100     20.409  35.144  58.139  1.00 0.00
      CCCC
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      CCCC
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      CCCC
ATOM  2316  H1  WAT  W   101     19.883  26.619  55.020  1.00 0.00
      CCCC
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      CCCC
ATOM  2318  OH2 WAT  W   102     59.643  24.389  43.849  1.00 39.53
      CCCC
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      CCCC
ATOM  2320  H2  WAT  W   102     59.793  25.086  43.222  1.00 0.00
      CCCC
ATOM  2321  OH2 WAT  W   103     60.566  27.542  41.229  1.00 46.40
      CCCC
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ATOM  2323  H2  WAT  W   103     60.987  27.015  41.915  1.00 0.00
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      CCCC
ATOM  2326  H2  WAT  W   104     15.858  29.940  55.028  1.00 0.00
      CCCC
      END
```

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by

the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Structure Determination

Crystals of native and selenomethione-substituted UCH-L3 were grown in space group $P2_12_12_1$ (a=48.6 Å, b=60.8 Å, c=81.4 Å). There is one molecule in the asymmetric unit and the solvent content is 48%. The structure of selenomethione-substituted UCH-L3 was determined at 2.35 Å resolution by the method of multiwavelength anomalous dispersion (MAD) (FIG. 2). The native structure was subsequently refined against 1.8 Å data to an Rvalue of 23.0% (free Rvalue = 28.6%) with good stereochemistry (RMSD bonds = 0.010 Å). The current refined UCH-L3 model contains 205 of the 230 residues. Three regions of UCH-L3 lack defined electron density and have been omitted from the model (residues 1-4, 147-166 and 218). The side chains of Arg-145 and Glu-203 also lack defined density and have been included in the model with occupancy of zero.

Structure of UCH-L3

UCH-L3 has overall dimensions of 43 Å × 32 Å × 37 Å. The structure is organized around a central six-stranded antiparallel β-sheet and two long α-helices. His-169 and Asp-184, which have both been implicated in catalysis, are located at the amino and carboxyl-terminal ends of strands 3 and strand 4 respectively. The right lobe includes a long buried α-helix (helix 4) which contains the active site nucleophile Cys-95, and a cluster of smaller helices. Helix 4 makes predominantly hydrophobic interactions with the β-sheet, several helices, and an extended segment. The active site of UCH-L3 is located between the molecule's two lobes, within a long cleft that appears to be closed in this unliganded structure. As discussed below, the catalytic nucleophile Cys-95, the general base His-169, and Asp-184, form a catalytic triad

that, along with other structural features, resembles the well known family of papain-like cysteine proteases (see FIG. 4).

A predicted secondary structure assignment was recently proposed for UCH-L3 and other UCH isozymes (Larsen *et al.*, 1996) using the neural network program of the PredictProtein server (Rost and Sander, 1993). This analysis predicted 34% α-helical content and 17% β-sheet for UCH-L3, which is similar to the observation of 37% α-helix and 20% β-sheet in the crystal structure. The PredictProtein server correctly predicted 5 out of 7 helices, and 3 out of 6 strands. However, a number of important secondary structural elements in the crystal structure are misidentified by the prediction, including helix 4, which contains the active site nucleophile, Cys-95, and strand 4, which terminates one residue before Asp-184, the third member of the catalytic triad.

Comparison with Other Structures

Although several well characterized classes of enzymes are known to have active site triads that apparently function to orient and activate either cysteine or serine nucleophiles, comparisons show that the papain family of cysteine proteases (Rawlings and Barrett, 1994), has great similarity with UCH-L3. The present inventors compared 21 papain-like structures that have been deposited in the Brookhaven database to UCH-L3 (FIG. 4 and FIG. 5). Of the papain-like structures, 3 are free enzyme, 4 have the active Cys bound either to oxygen atoms, 2-mercaptoethanol or metal ion, and 14 are inhibitor complexes; 13 are of papain, 4 cathepsin B, 3 actinidin, and 1 glycyl endopeptidase. Of the papain-like enzymes, cathepsin B has the structure with greatest overall similarity to UCH-L3 as indicated by a search performed with the Dali algorithm (Holm and Sander, 1993).

Overlap of the UCH-L3 active site triad (Cys-95, His-169, Asp-184) with the active site Cys, His, and Asn of the papain-like enzymes yields RMSD values on the three C^{α} atoms of between 0.07 Å and 0.32 Å for 21 papain-like structures in the

Brookhaven protein data base (FIG. 4). In addition, UCH-L3 Gln-89 is structurally equivalent to Gln-19 of papain, which participates in the formation of a catalytically important structure known as the oxyanion hole (Drenth *et al.*, 1976; Ménard *et al.*, 1991; Schröder *et al.*, 1993). Overlap of all four of these UCH-L3 active site residues on the papain-like enzymes yields RMSD values that range from 0.59 Å to 0.79 Å for C^{α} atoms, and from 0.84 Å to 1.2 Å for all atoms. Interestingly, the structural similarity extends to three buried water molecules of UCH-L3 that are located between the two lobes of the protein below the active site Cys and His. Two of these water molecules are also found in the papain-like enzymes, with the third site occupied by a serine side chain. It is possible that these conserved water molecules serve architectural roles to allow juxtaposition of the two lobes of the enzyme. It is also possible that they function in catalysis, either by facilitating conformational change (Rashin *et al.*, 1986) or substrate binding (Meyer *et al.*, 1988).

Structural similarity at the active sites suggests that the catalytic mechanism of UCHs will resemble that of the papain-like enzymes (Storer and Ménard, 1994). Thus, it is likely that UCH-L3 Cys-95 and His-169 form a thiolate/imidazolium ion pair, Asp-184 functions to orient the enzyme active site and perhaps to stabilize the protonated form of His-169, and Gln-89 contributes to the oxyanion hole. These roles in catalysis are consistent with mutagenesis data for the Cys, His, and Asp residues of UCH-L1 (Larsen *et al.*, 1996). In the unliganded structure, it appears unlikely that the Cys-95 side chain is deprotonated because the carbonyl oxygen atom of Ser-92 is positioned to form a linear 3.2 Å hydrogen bond with the Cys-95 thiol. It is likely that the thiolate ion will form after displacement of Ser-92, which, as discussed below, is expected to undergo conformational change upon substrate binding.

Starting from overlap on the active-site tetrad C^{α} atoms, optimal C^{α} superpositions of UCH-L3 with the papain-like enzymes were obtained using the program LSQMAN (Kleywegt and Jones, 1994). The best overlays were obtained with cathepsin B (Turk *et al.*, 1995) which shows 53 equivalent C^{α} atoms with a RMSD of 1.6 Å. The second best agreement is found with papain (Kamphuis *et al.*,

1984), which shows 39 equivalent C^{α} atoms and an RMSD of 1.2 Å. Superposition of UCH-L3 with papain on the 53 C^{α} atoms of the optimal UCH-L3/cathepsin B overlap resulted in an RMSD of 2.45 Å.

Segments of UCH-L3 that have structural equivalents in papain-like enzymes include most of the central antiparallel β-sheet, helix 4 (which contains the active site Cys), and an extended β-like segment adjacent to helix 4 (FIG. 5). The major difference between these structures is that the active site helix precedes the β-sheet in papain, while the active site helix is formed from the sequence following the second β-strand of the sheet in UCH-L3. This may have important functional consequences because it allows the positioning of a disordered loop of 20 residues over the active site of UCH-L3. As discussed below, this loop may play a role in substrate selection by the UCH enzymes.

A likely mode of substrate binding to UCH-L3 is suggested by analogy with complexes of papain-like enzymes, in which bound inhibitors occupy either the S or S′ sites (FIG. 6). (Substrate residues amino- and carboxyl-terminal to the scissile bond are designated P and P′ respectively, and the corresponding binding sites on the enzyme designated S and S′) (Schechter and Berger, 1967). The corresponding putative active-site cleft of UCH-L3 is closed by two short segments of the enzyme, which as described below, suggests a location to allow substrate binding. This proposed location for the UCH active site cleft is supported by the clustering of invariant surface-exposed residues in the region of the S site inhibitors of papain-like enzymes (FIG. 6C). This pattern of conserved residues is consistent with the very high specificity of UCH enzymes for ubiquitin, which is expected to bind to the proposed S sites, and the lack of selection for residues following ubiquitin, which are expected to bind in the proposed S′ sites.

Further insight on substrate binding is provided by the observation that UCH-L3 binds to ubiquitin with a micromolar dissociation constant and that this interaction has a significant electrostatic component (Larsen *et al.*, 1996). It is likely that the

positively charged basic face of ubiquitin (Wilkinson, 1988) will bind to UCH enzymes. Consistent with this idea, UCH-L3 has a molecular surface of almost entirely negative electrostatic potential (Nicholls *et al.*, 1991), including three invariant carboxylates (Glu-10, Glu-14, and Asp-33) at the putative S sites. As shown in FIG. 7, the present invention shows crudely docked ubiquitin against the proposed S sites of UCH-L3 so that electrostatic interactions appear favorable and the flexible C-terminal residues of ubiquitin are positioned analogously to the S site inhibitor of papain-like enzymes, with the ubiquitin C-terminus adjacent to the active site nucleophile, Cys-95. Hydrophobic surfaces on ubiquitin and UCH-L3 are also likely to contribute to the binding interaction.

Substrate Induced Conformational Changes

Comparison with ligand-bound complexes of papain-like enzymes suggests that the specificity of UCH enzymes for ubiquitin adducts may result, in part, from maintenance of an inactive enzyme conformation in the absence of a bound ubiquitin moiety. In the absence of a binding partner, the UCH-L3 active-site cleft appears to be closed by two loops (FIG. 8). The first of these loops includes Leu-9 and Glu-10, which are in van der Waals contact with groups on the opposite side of the cleft, and are in positions incompatible with the placement of papain-like enzyme inhibitors after least squares overlap on active site residues. It also seems likely that residues 11 and 12 will have to move in order to accommodate substrate. Interestingly, Glu-10 is one of the few surface exposed UCH residues that is invariant, and it is possible that binding of positively charged groups on ubiquitin to Glu-10 initiates opening of the UCH active site cleft.

The second loop that appears to block the active site, residues 90-94, spans the catalytic residues Gln-89 and Cys-95, and adopts a conformation that differs from the equivalent region of papain-like structures by displacements of more that 4 Å for the C^{α} atoms of residues 92 and 93. Consequently, the carbonyl oxygen of UCH-L3 Ser-92 is buried into the oxyanion hole in a position analogous to the oxygen atom of

inhibitors seen in the cysteine protease inhibitor/complex structures. The Ser-92 hydroxyl forms hydrogen bonding interactions with both the thiol and main chain amide of Cys-95. Because the adjacent residue, Asn-93, is both highly exposed and invariant, it is likely that this side chain may participate in substrate binding, thereby providing a mechanism to open the active site. Conformational change in both of the loops that appear to block the active site may be coupled since van der Waals contacts are observed from residue 9 to 93 and from 6 to 93 and 94.

Access to the active site appears to be further restricted by a 20 residue disordered loop consisting of residues 147 to 166 which spans the active site cleft. This loop may exist in several different conformations, and as discussed below, it is likely that it functions in the definition of substrate specificity. The observation of van der Waals contact between residues 7 and 146, and a hydrogen bonding interaction between residues 5 and 146 in the UCH-L3 crystal structure suggests the possibility of a coordinated conformational change upon substrate binding that includes the disordered loop.

Masking of the UCH active site in the absence of bound substrate may function to limit non-specific cleavages by these cytoplasmic proteases. An analogous conformational change probably does not occur for the papain-like enzymes. Inspection of the liganded and unliganded structures in the Brookhaven database shows no significant conformational changes in the enzyme S sites upon binding inhibitor. The papain-like enzymes, which are generally secreted or lysosomal, employ an alternative strategy to limit inappropriate reactions. Inhibitory N-terminal propeptide extensions are cleaved only after import into the lysosome (Carmona *et al.*, 1996; Coulombe *et al.*, 1996; Cygler *et al.*, 1996; Karrer *et al.*, 1993; Turk *et al.*, 1996).

Substrate Specificity

Although UCH-L3 has high specificity for ubiquitin N-terminal to the scissile bond, it is permissive for the residues following ubiquitin provided the adduct is small and unstructured. One possible rationale for the lack of activity against larger folded C-terminal ubiquitin fusions is that only highly extended substrates can be accommodated in a deep narrow groove of UCH S′ sites. The UCH-L3 crystal structure does not appear to possess such a groove, however, and thus the ordered protein visible in the crystal structure does not **obviously** explain the preference of UCH enzymes for small unfolded substrates. Although it is possible that a deep S′ site substrate cleft could be formed by conformation change upon binding to a substrate, the very low discrimination shown across a broad rage of sequences that are cleaved from the ubiquitin C-terminus argues against this possibility.

Specific UCH Active Site Modifications

More subtle modifications and changes may be made in the structure of the encoded UCH-L3 polypeptides of the present invention and still obtain a molecule that encodes a protein or peptide with characteristics of the natural UCH-L3 polypeptides, including the variants described above. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the following codon table, Table A:

Table A

Amino Acid Names and Abbreviations			Codons			
Alanine	Ala	A	GCA	GCC	GCG	GCU
Cysteine	Cys	C	UGC	UGU		
Aspartic acid	Asp	D	GAC	GAU		
Glutamic acid	Glu	E	GAA	GAG		

Table A (continued)

Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

It is known that certain amino acids may be substituted for other amino acids in a protein structure in order to modify or improve its antigenicity or activity (*e.g.*, Kyte and Doolittle, 1982; Hopp, U.S. Patent 4,554,101). For example, through the substitution of alternative amino acids, small conformational changes may be conferred upon a polypeptide which result in increased activity or stability. Alternatively, amino acid substitutions in certain polypeptides may be utilized to provide residues which may then be linked to other molecules to provide peptide-molecule conjugates which retain enough antigenicity of the starting peptide to be useful for other purposes. For example, a selected UCH-L3 peptide bound to a solid support might be constructed which would have particular advantages in diagnostic embodiments.

The importance of the hydropathic index of amino acids in conferring interactive biological function on a protein has been discussed generally by Kyte and Doolittle (1982), wherein it is found that certain amino acids may be substituted for other amino acids having a similar hydropathic index or core and still retain a similar biological activity. As displayed in Table B below, amino acids are assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics. It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant protein, which in turn defines the interaction of the protein with substrate molecules. Preferred substitutions which result in an antigenically equivalent peptide or protein will generally involve amino acids having index scores within ±2 units of one another, and more preferably within ±1 unit, and even more preferably, within ±0.5 units.

Table B

Amino Acid	Hydropathic Index
Isoleucine	4.5
Valine	4.2
Leucine	3.8
Phenylalanine	2.8
Cysteine/cystine	2.5
Methionine	1.9
Alanine	1.8
Glycine	-0.4
Threonine	-0.7
Tryptophan	-0.9
Serine	-0.8
Tyrosine	-1.3
Proline	-1.6
Histidine	-3.2
Glutamic Acid	-3.5
Glutamine	-3.5

Table B (continued)

Aspartic Acid	-3.5
Asparagine	-3.5
Lysine	-3.9
Arginine	-4.5

Thus, for example, isoleucine, which has a hydropathic index of +4.5, will preferably be exchanged with an amino acid such as valine (+ 4.2) or leucine (+ 3.8). Alternatively, at the other end of the scale, lysine (- 3.9) will preferably be substituted for arginine (-4.5), and so on.

Substitution of like amino acids may also be made on the basis of hydrophilicity, particularly where the biological functional equivalent protein or peptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with an important biological property of the protein.

As detailed in U.S. Patent 4,554,101, each amino acid has also been assigned a hydrophilicity value. These values are detailed below in Table C.

Table C

Amino Acid	Hydrophilic Index
arginine	+3.0
lysine	+3.0
aspartate	+3.0 ± 1
glutamate	+3.0 ± 1
serine	+0.3
asparagine	+0.2

Table C (continued)

glutamine	+0.2
glycine	0
threonine	-0.4
alanine	-0.5
histidine	-0.5
proline	-0.5 ± 1
cysteine	-1.0
methionine	-1.3
valine	-1.5
leucine	-1.8
isoleucine	-1.8
tyrosine	-2.3
phenylalanine	-2.5

It is understood that one amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

Accordingly, these amino acid substitutions are generally based on the relative similarity of R-group substituents, for example, in terms of size, electrophilic character, charge, and the like. In general, preferred substitutions which take various of the foregoing characteristics into consideration will be known to those of skill in the art and include, for example, the following combinations: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine (See Table D, below). The present invention thus contemplates functional or biological equivalents of an UCH-L3 or variant UCH-L3 polypeptide as set forth above.

Table D

Original Residue	Exemplary Substitutions
Ala	Gly; Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg
Met	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Biological or functional equivalents of a polypeptide can also be prepared using site-specific mutagenesis. Site-specific mutagenesis is a technique useful in the preparation of second generation polypeptides, or biologically functional equivalent polypeptides or peptides, derived from the sequences thereof, through specific mutagenesis of the underlying DNA. As noted above, such changes can be desirable where amino acid substitutions are desirable. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by Adelman, *et al.* (1983). As will be appreciated, the technique typically employs a phage vector which can exist in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage (Messing, *et al.*, 1981). These phage are commercially available and their use is generally known to those of skill in the art.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector which includes within its sequence a DNA sequence which encodes all or a portion of the UCH-L3 or variant UCH-L3 enzyme polypeptide sequence selected. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea *et al.* (1978). This primer is then annealed to the singled-stranded vector, and extended by the use of enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex

is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells such as *E. coli* cells and clones are selected which include recombinant vectors bearing the mutation. Commercially available kits come with all the reagents necessary, except the oligonucleotide primers.

In addition, peptides derived from these polypeptides, including peptides of at least about 6 consecutive amino acids from these sequences, are contemplated. Alternatively, such peptides may comprise about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 consecutive residues. For example, a peptide that comprises 6 consecutive amino acid residues may comprise residues 1 to 6, 2 to 7, 3 to 8 and so on of the UCH-L3 protein. Such peptides may be represented by the formula

x to (x + n) = 5′ to 3′ the positions of the first and last consecutive residues

where x is equal to any number from 1 to the full length of the UCH-L3 protein and n is equal to the length of the peptide minus 1. Where the peptide is 10 residues long (n = 10-1), the formula represents every 10-mer possible for each antigen. For example, where x is equal to 1 the peptide would comprise residues 1 to (1 + [10-1]), or 1 to 10. Where x is equal to 2, the peptide would comprise residues 2 to (2 + [10-2]), or 2 to 11, and so on.

Syntheses of peptides are readily achieved using conventional synthetic techniques such as the solid phase method (*e.g.*, through the use of a commercially available peptide synthesizer such as an Applied Biosystems Model 430A Peptide Synthesizer). Peptides synthesized in this manner may then be aliquoted in predetermined amounts and stored in conventional manners, such as in aqueous solutions or, even more preferably, in a powder or lyophilized state pending use.

In general, due to the relative stability of peptides, they may be readily stored in aqueous solutions for fairly long periods of time if desired, *e.g.*, up to six months or more, in virtually any aqueous solution without appreciable degradation or loss of antigenic activity. However, where extended aqueous storage is contemplated it will generally be desirable to include agents including buffers such as Tris or phosphate buffers to maintain a pH of 7.0 to 7.5. Moreover, it may be desirable to include agents which will inhibit microbial growth, such as sodium azide or Merthiolate. For extended storage in an aqueous state it will be desirable to store the solutions at 4°C, or more preferably, frozen. Of course, where the peptide(s) are stored in a lyophilized or powdered state, they may be stored virtually indefinitely, *e.g.*, in metered aliquots that may be rehydrated with a predetermined amount of water (preferably distilled, deionized) or buffer prior to use.

Of particular interest are peptides that represent antigenic epitopes that lie within the UCH-L3 polypeptides of the present invention. An "epitope" is a region of a molecule that stimulates a response from a T-cell or B-cell, and hence, elicits an immune response from these cells. An epitopic core sequence, as used herein, is a relatively short stretch of amino acids that is structurally "complementary" to, and therefore will bind to, binding sites on antibodies or T-cell receptors. It will be understood that, in the context of the present disclosure, the term "complementary" refers to amino acids or peptides that exhibit an attractive force towards each other. Thus, certain epitopic core sequences of the present invention may be operationally defined in terms of their ability to compete with or perhaps displace the binding of the corresponding UCH-L3 antigen to the corresponding UCH-L3 -directed antisera.

The identification of epitopic core sequences is known to those of skill in the art. For example U.S. Patent 4,554,101 teaches identification and preparation of epitopes from amino acid sequences on the basis of hydrophilicity, and by Chou-Fasman analyses. Numerous computer programs are available for use in predicting antigenic portions of proteins, examples of which include those programs based upon Jameson-Wolf analyses (Jameson and Wolf, 1988; Wolf *et al.*, 1988), the program

PepPlot® (Brutlag *et al.*, 1990; Weinberger *et al.*, 1985), and other new programs for protein tertiary structure prediction (Fetrow and Bryant, 1993) that can be used in conjunction with computerized peptide sequence analysis programs.

In general, the size of the polypeptide antigen is not believed to be particularly crucial, so long as it is at least large enough to carry the identified core sequence or sequences. The smallest useful core sequence expected by the present disclosure would be on the order of about 6 amino acids in length. Thus, this size will generally correspond to the smallest peptide antigens prepared in accordance with the invention. However, the size of the antigen may be larger where desired, so long as it contains a basic epitopic core sequence.

Small Molecule Inhibitors of UCH-L3 Variant Proteins

The present invention provides methods for screening and identifying small molecule inhibitors of UCH-L3 proteins and identifies such inhibitors. The rationale behind the design of the small molecule UCH-L3 protein inhibitors is that the structural differences between UCH-L3 proteins, caused by the deviations in the interatomic distances of the amino acid residues in the active site of the protein, will be exploited to design chemical ligands that bind to the active site of the different variant proteins to yield complexes with sufficient thermodynamic stability to effectively inhibit the functional activity of the protein. The inhibited UCH-L3 protein is thus unable to protect the tumor cell against the toxic action of the anticancer agent used to treat it. To obtain appropriate ligands that bind to the active sites of different UCH-L3 variant proteins, the inventors utilize the technique of forcefield docking of chemical fragments from both commercially available chemical fragment libraries, as well as in-house generated libraries, into the active electrophile-binding (H-) site in the derived crystal structure of each variant protein. The docked fragments will be energy-minimized and the binding energies computed and used to select candidate ligands.

Generation of UCH-L3 Inhibitors

Generation of inhibitors is accomplished by a rational drug development strategy involving force field docking and energy-minimization of chemical fragments and compounds into the active site of the variant UCH-L3 proteins. The compounds and chemical fragments can be drawn from chemical fragment libraries, such as that available in the Leapfrog database. Additional chemical libraries will be generated as necessary. The active site and other structural components of the variant UCH-L3 proteins will be derived from the published crystal structure of the UCH-L3 encoded protein.

One potential substitution that confers a functional change to the UCH-L3 protein is to replace cysteine 95 with a serine that, in context with other such changes, results in a protein that is more chemically stable and resistant to oxidation and heat. Other proposed changes in this context include substituting aspartic acid 184 for asparagine. Moreover, it is recognized that leucine may be substituted for methionine, or a serine or alanine may be substituted for cystine to result in increased stability. Increased protein stability also results from the addition of disulfide bonds and the creation of more hydrophobic interactions within the protein structure.

Based on the resultant DDH values obtained after energy minimization of chemical fragments/compounds, candidate inhibitors are selected and/or newly constructed from chemical fragments for synthesis and further analyses for their inhibitory or other action on the variant UCH-L3 proteins. Selection criteria for inhibitors for synthesis and further analysis includes lipophilicity, chemical stability and availability or ease of synthesis.

Candidate inhibitors of the present invention may include such molecules as substituted, heterocyclic aromatic compounds, sugar-linked aromatic compounds and other aromatic compounds.

The substituted groups may vary between the different compounds and result in significant changes in binding energies of the compounds in the active site pocket of the UCH-L3 protein. For example, R_1 substitutions of either NH_2 or OH, cause changes in binding energies of almost 10 kcals/mol. Other important substitutions are the alkyl or aminoalkyl substitutions of R_3, and the alkyl, phenyl or 2-pyridyl substitutions of R_4, some of which result in changes in binding energies of greater than 10 kcals/mol.

However it is conceivable that any of the R groups of the substituted isoxazoles may be a phenyl group, a benzyl group, an aryl group, an alkyl group, an aryl group linked to another aryl group through an ester linkage, an aryl group linked to an alkyl group with an ester linkage, an aryl group linked to another aryl group through an ether linkage and aryl group linked to an alkyl group with a thiolester linkage, an alkyl group linked to another alkyl group through an ester linkage, an alkyl group linked to another alkyl group through an ether linkage, an alkyl to alkyl linked through an amino group, an aryl to alkyl linked through an amino group. an alkyl group through a disulphide group, an aryl linked to an alkyl group through a disulphide group, an aryl linked to another aryl group through a disulphide group, an alkyl linked to another alkyl group through a thioester linkage, an aryl linked to an alkyl group through a polyester linkage, an aryl group linked to another aryl through a polyester linkage, an alkyl group linked to another alkyl group through a polyamine linkage, an aryl linked to an alkyl group through a polyamine linkage, an aryl group linked to another aryl through a polyamine linkage, an alkyl group linked to another alkyl group through a polythioester linkage, an aryl linked to an alkyl group through a polythioester linkage, an aryl group linked to another aryl through a polythioester linkage.

An individual skilled in the art of organic synthesis in light of the present disclosure is able to prepare or identify a large variety of substituted isoxazoles which would be expected to have UCH-L3 inhibitory effects in the light of the present disclosure.

Screening for Modulators of UCH-L3.

Within certain embodiments of the invention, methods are provided for screening for modulators of UCH-L3 protein activity. Such methods may use labeled UCH-L3 proteins or analogs, anti-UCH-L3 proteins or anti-UCH-L3 antibodies and the like as reagents to screen small molecule and peptide libraries to identify modulators of UCH-L3 protein activity. Within one example, a modulator screening assay is performed in which cells expressing UCH-L3 proteins are exposed to a test substance under suitable conditions and for a time sufficient to permit the agent to effect activity of UCH-L3 proteins .

Assay for Ubiquitin Carboxy Terminal Hydrolase Activity

To perform the assay, purified UCH-L3 or variant UCH-L3 peptide is diluted into 10 mM dithiothreitol (DTT) and allowed to preincubate on ice for 1 h. The standard assay contains 12 μM UbOEt (ubiquitin carboxy-terminal ethyl ester), 100 mM potassium phosphate, pH 7.2 (37°C), 10 mM dithiothreitol, 0.2 mM EDTA, and enzyme diluted to a final concentration of 0.4 mIU/ml. The reaction is incubated at 37°C and aliquots containing 1-2 g total ester plus hydrolysis product are withdrawn at ten minute intervals and immediately injected onto an HPLC column (C-8, 5 micron, 4 mm × 250 mm; Altech Associates, Deerfield, IL), flow rate of 1 ml/min in a solvent comprising 25 mM sodium Perchlorate and 0.07% (v/v) perchloric acid in 49% HPLC grade acetonitrile. The absorbance at 205 nm is monitored and the resulting peaks are quantitated by manual integration of the areas.

Measurement of Deconjugating Activity

^{125}I-ubiquitin was synthesized by the chloramine-T method (Ciechanover *et al.* 1978, Biochem. Biophys. Res. Comm. 81, 1100-1104). ^{125}I-ubiquitin was conjugated to the proteins of reticulocyte fractions by incubating the following in a final volume of 0.8 ml: 2 mg/ml of proteins, 3 μg/ml ^{125}I-ubiquitin (1.2×10^6

cpm/μg), 50 mM Tris HCl, pH 7.6, 1 mM magnesium chloride, 0.4 mM ATP, 0.4 mM DTT, 2 mM phosphocreatine, 3 units creatine phosphokinase, and 0.1 mM hemin. After incubating 2 h at 37°C, iodoacetamide was added to a final concentration of 10 mM, and was allowed to react for 30 min. at 37°C. Dithiothreitol was then added to a concentration of 50 mM to quench the alkylating agent, and the mixture was chromatographed on a Sephadex G-50 column (1.5 cm × 60 cm) equilibrated with 50 mM ammonium acetate. The fractions containing ^{125}I-ubiquitin were located by gamma counting, and the counts in the exclusion volume are pooled. The percentage of ubiquitin incorporated into high molecular weight complexes is about 10%, and this fraction is utilized in deconjugation assays.

To measure deconjugation, the ^{125}I-ubiquitin conjugates are incubated with 0.01U of the UCH fraction in 50 mM Tris HCl pH 8.0, 0.1 mM EDTA, and 10 mM DTT. After 30 min. or 2 h, the reaction is terminated by the addition of two parts reaction mixture to one part 9% SDS, 15% glycerol, 0.2 M Tris Hcl, pH 6.8, and 3 mM EDTA. The samples are then subjected to SDS-PAGE according to standard techniques. The resulting gels dried and sliced into strips. The molecular weight distribution of ^{125}I-ubiquitin is determined by gamma counting of the gel slices. Rates of deconjugation is calculated by the fraction of counts appearing in the sub-10 kD region relative to the entire lane.

Generally the test substance is added in the form of a purified agent, however it is also contemplated that test substances useful within the invention may include substances present throughout the handling of test sample components, for example host cell factors that are present in a cell lysate used for generating the test sample. Such endogenous factors may be segregated between the test and control samples for example by using different cell types for preparing lysates, where the cell type used for preparing the test sample expresses a putative test substance that is not expressed by the cell type used in preparing the control sample.

The active compounds may include fragments or parts of naturally-occurring compounds or may be only found as active combinations of known compounds which are otherwise inactive. However, prior to testing of such compounds in humans or animal models, it may be necessary to test a variety of candidates to determine which have potential.

Accordingly, in screening assays to identify agents which alter the activity of UCH-L3 proteins in for example cancer cells, it is proposed that compounds isolated from natural sources, such as animals, bacteria, fungi, plant sources, including leaves and bark, and marine samples may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds.

In these embodiments, the present invention is directed to a method for determining the ability of a candidate substance to decrease the UCH-L3 activity of cancer cells, the method including generally the steps of:

(a) obtaining a cell with UCH-L3 activity;

(b) admixing a candidate substance with the cell; and

(c) determining the ability of the candidate substance to inhibit the UCH-L3 activity of the cell.

To identify a candidate substance as being capable of decreasing UCH-L3 activity, one would measure or determine the basal UCH-L3 status of for example a cancer cell prior to any additions or manipulation. One would then add the candidate substance to the cell and re-determine the UCH-L3 activity in the presence of the candidate substance. A candidate substance which decreases the UCH-L3 activity relative to the composition in its absence is indicative of a candidate substance being an inhibitor of UCH-L3.

The candidate screening assay is quite simple to set up and perform, and is related in many ways to the assay discussed above for determining UCH-L3 content.

"Effective amounts", in certain circumstances, are those amounts effective at reproducibly decrease UCH-L3 activity in an assay in comparison to their normal levels. Compounds that achieve significant appropriate changes in activity will be used. If desired, a battery of compounds may be screened *in vitro* to identify other agents for use in the present invention.

A significant decreases in UCH-L3 activity, are represented by a decrease in UCH-L3 protein activity levels of at least about 30%-40%, and most preferably, by decreases of at least about 50%, with higher values of course being possible. Assays that measure UCH-L3 activity in cells are well known in the art and may be conducted *in vitro* or *in vivo*, and have been described elsewhere in the specification.

Quantitative *in vitro* testing of the UCH-L3 inhibitors is not a requirement of the invention as it is generally envisioned that the agents will often be selected on the basis of their known properties or by structural and/or functional comparison to those agents already demonstrated to be effective. Therefore, the effective amounts will often be those amounts proposed to be safe for administration to animals in another context.

EXAMPLE 1: SMALL MOLECULE INHIBITORS OF UCH-L3 AND UCH-L3 VARIANTS

1. Materials and Methods

Generation of UCH-L3 inhibitors. Generation of inhibitors is accomplished by a rational drug development strategy involving force field docking and energy-minimization of chemical fragments and compounds into the active site of the variant UCH-L3 proteins. The compounds and chemical fragments can be drawn from chemical fragment libraries, such as that available in the Leapfrog database.

Additional chemical libraries will be generated as necessary. The active site and other structural components of the variant UCH-L3 proteins will be derived from the published crystal structure of the UCH-L3 encoded protein. Selection criteria for inhibitors for synthesis and further analysis includes lipophilicity, chemical stability and availability or ease of synthesis.

Synthesis of UCH-L3 Inhibitors. If the identified and/or newly constructed potential inhibitors are not commercially available, then they will be synthesized using standard organic synthetic methodology, including heterocyclic ring construction and functionalization, and electrophilic and nucleophilic substitution reactions. Reaction mixtures will be separated by thin layer, flash silica gel column and high performance liquid chromatography (TLC, CC and HPLC). The compounds will be purified using standard techniques modified as necessary. Characterization of synthetic products will be done by melting point determination, Fourier transform infrared (FT-1R), ultraviolet (UV) and high resolution nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. Compounds for biological testing will be purified by preparative HPLC. The purity of compounds will be determined by elemental analysis and HPLC.

Source of variant UCH-L3 proteins. To examine the ability of the inhibitors selected from the rational design described above to inhibit the variant UCH-L3 proteins, the present invention will utilize recombinant UCH-L3 proteins expressed in *E. coli* transfected with expression vectors containing the corresponding cDNAs. These vectors have been described elsewhere in this application. The UCH-L3 proteins will be purified by GSH-affinity chromatography on S-hexyl glutathione linked to epoxy-activated sepharose 6B. and then used for enzyme kinetic analysis.

It is also recognized that one may employ a ubiquitin affinity column to purify the UCH-L3 proteins and their variants. In this method, UCH-L3 and or variant UCH-L3 is contacted with activated CH-Sepharose 4B to which ubiquitin is bound. The enzyme forms a thiol ester linkage to the bound ubiquitin in the presence of ATP

and is eluted with AMP plus inorganic pyrophosphate. The amount of functional enzyme is determined from the counts of (^{3}H)ATP made acid insoluble by formation of 1 enzyme equivalent of (^{3}H)AMP-ubiquitin. Treatment of the activating enzyme with iodoacetamide renders it unable to form $E_{S\text{-}ubiquitin}$ but has no effect on formation of E-AMP-ubiquitin (Ross and Warms, *Biochemistry*, 22:4234-4237 (1983).

Analysis of inhibitors for UCH-L3 inhibitory activity. These studies will be performed using standard enzyme kinetic methodologies. The purified variant UCH-L3 proteins will be mixed with increasing inhibitor concentrations and at different time points, residual deubiquitinating activity will be determined as set forth above.

Synthesis of Isoxazoles. Using the techniques described above, potential UCH-L3 inhibitors such as isoxazoles have been identified. In the synthetic strategy for obtaining isoxazole deubiquitinating inhibitors, the ring system can be achieved by the usual approach of cyclization between hyroxylamine and three-carbon atom component such as 1,3-diketone or an a,b-unsaturated ketone or by a 1,3-dipolar cycloaddition reaction involving nitride oxides with alkenes or an alkyne (Glichrist, 1992).

The first class of compounds are substituted isoxazoles, with the general structure shown in structures 1-3. The substituted groups in the different compounds are represented by R_1, R_2, R_3, and R_4. The substituted groups vary between the different compounds and result in significant changes in binding energies of the compounds in the active site pocket of the UCH-L3 protein. For example, R_1 substitutions of either NH_2 or OH, cause changes in binding energies of almost 10 kcals/mol. Other important substitutions are the alkyl or aminoalkyl substitutions of R_3, and the alkyl, phenyl or 2-pyridyl substitutions of R_4, some of which result in changes in binding energies of greater than 10 kcals/mol.

Another group of potential variant UCH-L3 protein inhibitors identified by the strategy described in this invention are the heterocyclic aromatic compounds. The

binding energies range from -34 to -94 kcal/mol, depending upon the type of compound or substitution.

Antibodies

Antibodies to UCH-L3 or UCH-L3 variant peptides or polypeptides may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Patent 4,196,265. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, *e.g.*, purified or partially purified protein, synthetic protein or fragments thereof, as discussed in the section on polypeptides. Animals to be immunized are mammals such as cats, dogs and horses, although there is no limitation other than that the subject be capable of mounting an immune response of some kind. The immunizing composition is administered in a manner effective to stimulate antibody producing cells. Rodents such as mice and rats are preferred animals, however, the use of rabbit, sheep or frog cells is possible. The use of rats may provide certain advantages, but mice are preferred, with the BALB/c mouse being most preferred as the most routinely used animal and one that generally gives a higher percentage of stable fusions.

For generation of monoclonal antibodies (MAbs), following immunization, somatic cells with the potential for producing antibodies, specifically β lymphocytes (β cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens, tonsils or lymph nodes, or from a peripheral blood sample. Spleen cells and peripheral blood cells are preferred, the former because they are a rich source of antibody-producing cells that are in the dividing plasmablast stage, and the latter because peripheral blood is easily accessible. Often, a panel of animals will have been immunized and the spleen of the animal with the highest antibody titer removed. Spleen lymphocytes are obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

The antibody-producing B cells from the immunized animal are then fused with cells of an immortal myeloma cell line, generally one of the same species as the animal that was immunized. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells, called "hybridomas."

Any one of a number of myeloma cells may be used and these are known to those of skill in the art. For example, where the immunized animal is a mouse, one may use P3-X63/Ag8, X63-Ag8.653, NS1/1.Ag 41, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with human cell fusions.

One preferred murine myeloma cell line is the NS-1 myeloma cell line (also termed P3-NS-1-Ag4-1), which is readily available from the NIGMS Human Genetic Mutant Cell Repository by requesting cell line repository number GM3573. Another mouse myeloma cell line that may be used is the 8-azaguanine-resistant mouse murine myeloma SP2/0 non-producer cell line.

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Gefter *et al.* (1977). The use of electrically induced fusion methods is also appropriate.

Fusion procedures usually produce viable hybrids at low frequencies, from about 1×10^{-6} to 1×10^{-8}. This does not pose a problem, however, as the viable, fused hybrids are differentiated from the parental, unfused cells (particularly the unfused myeloma cells that would normally continue to divide indefinitely) by culture in a selective medium. The selective medium generally is one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented with hypoxanthine.

The preferred selection medium is HAT. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

The selected hybridomas are then serially diluted and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to

provide MAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected, usually in the peritoneal cavity, into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography.

Monoclonal antibodies of the present invention also include anti-idiotypic antibodies produced by methods well-known in the art. Monoclonal antibodies according to the present invention also may be monoclonal heteroconjugates, *i.e.*, hybrids of two or more antibody molecules. In another embodiment, monoclonal antibodies according to the invention are chimeric monoclonal antibodies. In one approach, the chimeric monoclonal antibody is engineered by cloning recombinant DNA containing the promoter, leader, and variable-region sequences from a mouse antibody producing cell and the constant-region exons from a human antibody gene. The antibody encoded by such a recombinant gene is a mouse-human chimera. Its antibody specificity is determined by the variable region derived from mouse sequences. Its isotype, which is determined by the constant region, is derived from human DNA.

In another embodiment, monoclonal antibodies according to the present invention is a "humanized" monoclonal antibody, produced by techniques well-known in the art. That is, mouse complementary determining regions ("CDRs") are transferred from heavy and light V-chains of the mouse Ig into a human V-domain, followed by the replacement of some human residues in the framework regions of their murine counterparts. "Humanized" monoclonal antibodies in accordance with

this invention are especially suitable for use in *in vivo* diagnostic and therapeutic methods for treating *Moroxella* infections.

As stated above, the monoclonal antibodies and fragments thereof according to this invention can be multiplied according to *in vitro* and *in vivo* methods well-known in the art. Multiplication *in vitro* is carried out in suitable culture media such as Dulbecco's modified Eagle medium or RPMI 1640 medium, optionally replenished by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements, *e.g.*, feeder cells, such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages or the like. *In vitro* production provides relatively pure antibody preparations and allows scale-up to give large amounts of the desired antibodies. Techniques for large scale hybridoma cultivation under tissue culture conditions are known in the art and include homogenous suspension culture, *e.g.*, in an airlift reactor or in a continuous stirrer reactor or immobilized or entrapped cell culture.

Large amounts of the monoclonal antibody of the present invention also may be obtained by multiplying hybridoma cells *in vivo*. Cell clones are injected into mammals which are histocompatible with the parent cells, *e.g.*, syngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as Pristane (tetramethylpentadecane) prior to injection.

In accordance with the present invention, fragments of the monoclonal antibody of the invention can be obtained from monoclonal antibodies produced as described above, by methods which include digestion with enzymes such as pepsin or papain and/or cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present invention can be synthesized using an automated peptide synthesizer, or they may be produced manually using techniques well known in the art.

The monoclonal conjugates of the present invention are prepared by methods known in the art, *e.g.*, by reacting a monoclonal antibody prepared as described above with, for instance, an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents, or by reaction with an isothiocyanate. Conjugates with metal chelates are similarly produced. Other moieties to which antibodies may be conjugated include radionuclides such as ^{3}H, ^{125}I, ^{131}I ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, and ^{99}mTc, are other useful labels which can be conjugated to antibodies. Radioactively labeled monoclonal antibodies of the present invention are produced according to well-known methods in the art. For instance, monoclonal antibodies can be iodinated by contact with sodium or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Monoclonal antibodies according to the invention may be labeled with technetium-^{99}m by ligand exchange process, for example, by reducing pertechnate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column or by direct labeling techniques, *e.g.*, by incubating pertechnate, a reducing agent such as $SNCl_2$, a buffer solution such as sodium-potassium phthalate solution, and the antibody.

The present invention contemplates that the exclusion of large ubiquitin fusions from the UCH-L3 active site results from the 20 residue loop between Thr-147 and Val-166 that is disordered in the instant crystals. This loop is topologically distinct from the papain-like enzymes. The ends of the loop are anchored 20 Å apart on opposite sides of the active site Cys-95 and three different classes of conformations can be envisioned for the loop with respect to the proposed UCH-substrate interaction geometry (FIG. 9).

The loop may be sandwiched between the body of UCH-L3 and the ubiquitin moiety of a substrate (red conformation in FIG. 9). This arrangement seems unlikely, however, in light of the probable ubiquitin binding surface on UCH-L3 (see above). Furthermore, the loop sequence is not well conserved, and thus seems poorly suited to

mediate interactions with ubiquitin, for which all UCH enzymes that have been characterized exhibit high specificity.

A second possible conformation places the loop over the active site, with residues C-terminal to the scissile bond passing through the loop (blue in FIG. 9). When modeled in a maximally open conformation the loop has an internal diameter of approximately 15 Å, which is suitable for passage of an unfolded extended polypeptide chain, although it is expected to limit passage of even a small folded structure such as an α-helix. A problem with this model is that the *D. melanogaster* UCH is able to cleave ubiquitin from conjugates with the large substrate IκBα (Roff *et al.*, 1996), and that the *S. cerevisiae* UCH cleaves conjugates from cytochrome c (Cohen).

Alternatively, the disordered loop may fold completely away from the proposed ubiquitin-binding surface (magenta in FIG. 9). This conformation would be analogous to the occluding loop of cathepsin B, which is also located along the S′ sites and defines the exopeptidase specificity of cathepsin B by making specific interactions with the substrate carboxyl terminus two residues beyond the scissile bond (Turk *et al.*, 1995). An important topological distinction is that, unlike the disordered loop of UCH-L3, the cathepsin B occluding loop does not straddle the active cleft site (in FIG. 5A the occluding loop partially obscures the active site Gln, Cys, and His of cathepsin B).

The present invention contemplates changing the topology of the UCH-L3 protein to make it more papain-like in structure, such that the resulting protein is capable of cleaving peptides as well as larger proteins from ubiquitin. In constructing such a molecule, the disordered loop that straddles the active site is reduced or eliminated, thus opening the active site.

It is possible that upon binding of ubiquitin adducts, the disordered loop will remain mobile, fluctuating between the extreme magenta and blue conformations of

FIG. 9. Thus, the loop will impede active site access for a wide range of larger substrates, which may eventually attain a productive complex by using either the blue or magenta conformations. It is also possible that the disordered loop plays a more active role in the selection of substrates *in vivo*, perhaps even becoming ordered and contributing directly to binding of some physiological substrates. This model suggests the intriguing possibility that the disordered loops of the different UCH enzymes, which are of similar length but relatively dissimilar sequence identities, function as modular units to confer different substrate specificity on the various UCH isozymes.

Regulating protein degradation by regulating protein deubiquitination can be stimulating or inhibiting degradation. Where protein degradation is to be stimulated a protein whose degradation is ubiquitin-dependent is exposed to a UCH-L3 or mutant UCH-L3 enzyme of the present invention.

Where protein degradation is to be inhibited, a protein whose degradation is ubiquitin-dependent is exposed to a mutant deubiquitinating enzyme of the present invention, which mutant does not catalyze the deubiquitination of proteins.

Exposing can be accomplished *in vitro* or *in vivo*. *In vitro* deubiquitinating processes have application in the industrial bulk production of proteins such as enzymes. A deubiquitinating enzyme of the present invention can be used in such processes to remove ubiquitin from the produced protein or to direct the removal of selected terminal amino acid residues. The use of deubiquitinating enzymes for generating desired amino-terminal residues of proteins is described in United States Patent No. 5,093,242, the disclosure of which is incorporated herein by reference.

Where exposing is accomplished *in vivo*, cells lacking an endogenous deubiquitinating system or cells having a mutation or deficiency in a deubiquitinating enzyme are transfected with a polynucleotide comprising a DNA sequence that encodes a deubiquitinating enzyme. Alternatively, a cell can be transfected with an

expression vector comprising a DNA sequence that encodes a mutant UCH-L3 such that the natural protein degradation pathway for a protein is inhibited.

Processes for destabilizing proteins *in vivo*, producing proteins using ubiquitin fusion and the *in vitro* cleavage of ubiquitin fusion proteins are well known in the art. Descriptions of such processes can be found in United States Patent Nos. 5,122,463, 5,132,213 and 5,196,321, the disclosures of which are incorporated herein by reference. In addition, the nucleotide and amino acid residue sequences of ubiquitin-specific proteases can be found in United States Patent No. 5,212,058, the disclosure of which is incorporated herein by reference.

EXAMPLE 2: SPECIFICITY AND *IN VIVO* ROLES OF UCH ISOZYMES

To further define the specificity and the *in vivo* roles of UCH isozymes, the present inventors tested natural and semi-synthetic ubiquitin derivatives as substrates, with specific emphasis on their potential role in ubiquitin proprotein and polyubiquitin processing. The results suggest that human UCH isozymes L1 and L3 are apparently involved in processing of proubiquitin gene products and small molecular weight ubiquitin adducts, but not larger derivatives of ubiquitin.

Procedures

Materials

Ubiquitin C-terminal hydrolases were prepared as described previously (Larsen *et al.*, 1996). All chemicals were reagent grade or better. Restriction endonucleases and DNA modification enzymes were from New England Biolabs, Beverly, MA. Recombinant human ubiquitin was expressed in *E. coli* and purified as described below.

Subcloning of proprotein genes

The human UbCEP52 and UbCEP80 and the *S. cerevisiae ubi4* proubiquitin genes were excised from pSP72 cloning vector (Monia *et al.*, 1989) by digestion with *Eco*RV and *Kpn*I. The cassette was ligated to a 5' *Nde*I site (Klenow polymerase blunted) and the 3' *Kpn*I site of the prokaryotic expression vector pRSET B (Invitrogen) with T4 DNA ligase. After transformation of the ligation mixture to Top 10 F' competent *E. coli* (Invitrogen), clones were grown for DNA miniprep and assayed by restriction digestion with *Sca*I and *Xho*I (UbCEP52) or *Sca*I and *Bam*HI (UbCEP80). Correct recombinant plasmids were amplified and stored at -20°C in TE buffer (Sambrook *et al.*, 1989). The yeast proubiquitin gene was similarly inserted into the Klenow-blunted pRSET using *Eco*RV and *Hin*dIII, and colonies were screened with by *Xho*I restriction digests of the isolated plasmids. These ubiquitin proprotein expression plasmids were named pRSUb52, pRSUb80, or pRSyUb5, respectively.

Purification of ubiquitin proproteins

The *E. coli* host strain BL21(DE3) (Invitrogen) was transformed with the appropriate expression vectors described above. For Ub-CEP proteins, the strain BL21(DE3)pLysE was used. Individual colonies were inoculated into 200 ml LB media (Sambrook *et al.*, 1989) supplemented with ampicillin (50 μg/ml) and grown overnight at 37°C. This culture was used to inoculate 2 or 12 liters of LB media. When the optical density (600 nm) of the cultures reached 0.45 (UbCEP) or 0.6 (yUb5), IPTG was added to 0.3 mM, and the cultures were grown for an additional 3 h. The cells were pelleted at 4000 RPM in an RC-3 rotor. Lysozyme was added to 0.1 mg/ml, and the bacteria were incubated for thirty minutes at 37°C, sonicated, and recentrifuged as above. UbCEP52 was purified from the supernatant as described previously (Monia *et al.*, 1989), with additional purification over a 300 ml sephadex G-75SF gel filtration column and MonoS FPLC (Pharmacia)

Recombinant yeast proubiquitin was expressed in *E. coli* and purified by a modification of Jonnalagadda *et al.* (1987). The bacteria were harvested by centrifugation, suspended in 50 mM Tris-Cl, pH 7.8, 1 mM EDTA, and sonicated (Heat Systems). After centrifugation for thirty minutes at 15,000 × *g*, the supernatant was raised to 65°C for five minutes, and centrifuged again as above. The resulting heat stable supernatant was made 85% saturated in ammonium sulfate, stirred gently overnight at 4°C, and was centrifuged for thirty minutes at 10,000 × *g* in a GSA rotor. The pellet was resolubilized in a minimal volume of water, and after lowering its pH to 4.6 with 1 M acetic acid, was applied to an FPLC Mono S 5/5 column (Pharmacia) in 50 mM NaOAc pH 4.5. Ubiquitin oligomers were eluted in a linear gradient of 0 to 550 mM NaCl. Oligomers which cross reacted with anti-ubiquitin polyclonal antibodies (Accurate Scientific) eluted at 150, 200, 290, 350, and 400 mM NaCl (n= 1 to 5 ubiquitins respectively). The pooled fractions were dialyzed against 10 mM Tris-Cl, pH 7.6, concentrated by ultrafiltration. The preparation was homogeneous as judged by coomassie-stained SDS-PAGE.

Purification of truncated ubiquitin gene products

To study P′ specificity, the truncated ubiquitin gene products, Ub-CEP52$^{1-10}$, Ub-CEP80$^{1-10}$, and Ub-Ub$^{1-10}$ were prepared. Vectors encoding ubiquitin fused to the first ten residues of CEP52 (Ub-IIEPSLRQLA) **(SEQ ID NO:1)**, CEP80 (Ub-GKKRKKKVYT) **(SEQ ID NO:2)**, or Ub (Ub-MQIFVKTLTG) **(SEQ ID NO:3)**. Bacteria harboring the expression plasmids were grown to an A_{600} of 0.6, and induced for protein production with 0.5 mM IPTG. Supernatants were made as above, but with 10 mM DTT in the buffer. The supernatants containing Ub-CEP52$^{1-10}$ or the Ub-CEP80$^{1-10}$ were heat treated at 86°C for five minutes, cooled to 4°C, and centrifuged at 3,500 × *g* for 15 min. In most cases, the supernatant was chromatographed on a 1 liter column of G-100 superfine (Pharmacia). The supernatant containing Ub-Ub$^{1-10}$ was pretreated with 2.5% perchloric acid and centrifuged. The acid-soluble supernatant was subjected to gel filtration as above. In

all cases, the fusion proteins obtained were homogeneous as judged by Coomassie-stained SDS-PAGE.

Preparation of Ub-amino acid extension proteins

A vector library encoding a variety of single amino acids C-terminal to ubiquitin was constructed using the polymerase chain reaction. To create this amino acid library at position 77, the coding region of the pRSUb80 vector (see above) was amplified with a degenerate 3′ primer which contained all possible codons followed by a stop codon and a *Hin*dIII site. The primer sequences were: 5′-ATCCATATGCAGATCTTCG-3′ **(SEQ ID NO:4)**, and 5′-CAAGCTTCTANNNACCACCACGAAGTC-3′ **(SEQ ID NO:5)**. The PCR™ products from this reaction were subcloned *en masse* into pCRII (Invitrogen, San Diego, CA), and 40 minipreps were prepared. Inserts were present in 25 of the 40 minipreps and these inserts were sequenced (Sanger *et al.*, 1977). Clones were identified which encoded D, H, K, P, S, or T at the C-terminus. These were subcloned into pRSET using their *Nde*I and *Hin*dIII sites. Proteins were expressed and purified by heat denaturation and gel filtration, as described above. One additional clone was recovered due to a deletion in the PCR™ product. This frameshift resulted in a vector encoding Nα-ubiquitinyl-PRSLDSC, which was also expressed and purified.

Co-translational processing

The kanamycin resistance gene was incorporated into plasmids encoding UCH-L1 or UCH-L3 by insertion of a DNA cassette from pUC4K (Pharmacia). pRSUCH plasmids were digested with *Eco*RI and calf intestinal phosphatase. The kan^r gene cassette was excised from pUC4K with *Eco*RI. After gel purification of the insert and vector fragments, they were ligated and plated onto LB-kanamycin agar plates. The correct transformants were identified by the presence of a unique *Sca*I site in the kan^r cassette, and the amp^r gene was subsequently disabled by excision of an AvaII fragment in its center, followed by religation.

To co-express enzymes and putative substrates in the same cell, BL21(DE3) cells harboring either the pRSyUb5, the pRSUb52, or the pRSUb80 plasmid (amp^r) were transformed with a pRSUCH plasmid (kan^r) and plated on LB agar containing both kanamycin and ampicillin to select for co-transformants. Induction with IPTG resulted in co-expression of the selected UCH isozyme along with a putative substrate. Processing was assessed by adding SDS-PAGE sample buffer directly to cell pellets and analyzed by Western blotting using antibodies specific for each substrate.

Other substrates

A plasmid encoding a Ub-R-β-galactosidase fusion protein was obtained from Dr. Alex Varshavsky (pKKUbRβGal). Synthesis of Ub-R-β-gal was induced as described previously, and the fusion protein was purified as described for UCH, except that the anion exchange resin was eluted with 50 mM Tris-HCl, pH 7.5 and containing 150 mM NaCl. This resulted in significantly purified protein preparation (>80% homogeneous) which was used in gel and HPLC assays of fusion protein processing.

K48-linked diubiquitin (Nε-Ubiquitinyl-^{K48}Ub) was synthesized *in vitro* by incubation of human recombinant or bovine ubiquitin (Sigma, St. Louis, MO) with the activating and conjugating enzymes of the ubiquitin system (Chen and Pickart, 1990). Incubations contained 50 mM Tris-Cl pH 8.0, 2 mM ATP, 5 mM $MgCl_2$, 5 mM phosphocreatine, 0.3 units/ml phosphocreatine kinase, 0.3 U/ml inorganic pyrophosphatase, 10 μg/ml ovalbumin, 30 μM E2-25k (plasmid obtained from Cecile Pickart), 0.1 μM E1 from rabbit liver (A. L. Haas), and 5 to 10 mg/ml ubiquitin. Reaction mixtures were incubated at 37°C for 40 min. The E1 and E2 enzymes were removed by passing the reaction mixture over a Mono Q anion exchange column (Pharmacia) at pH 7.6. Polyubiquitin chains were purified by chromatography on Mono S FPLC (Pharmacia) as described above for proubiquitin.

As used herein, the term "polyubiquitin chains" or "polymeric ubiquitin derivatives" are named as follows. The polyprotein ubiquitin gene product (UBI4p in yeast) is referred to as proubiquitin. The products of the UBI1, 2 and 3 genes in yeast are referred to as ubiquitin C-terminal extension proteins (UbCEP). The length of the CEP can be added as a suffix; *i.e.* UbCEP52 or UbCEP76 in yeast. When the C-terminal carboxyl group of ubiquitin is involved in an amide bond, it is referred to as the ubiquitinyl group (Ub). The amino component of this amide bond can be contributed by either the amino terminus of a peptide (Nα-ubiquitinyl-peptide) or the ε-amino group of lysine (Nε-ubiquitinyl-lysine). Where known, the number of the specific lysine in a peptide can be specified as a superscript prefix. Thus, a K48 linked ubiquitin dimer is referred to as Nε-ubiquitinyl-^{K48}Ub. A larger polymer of ε-linked ubiquitin is referred to as polyubiquitin, with the identity of the specific lysine involved specified as a superscript prefix (*i.e.* ^{K48}polyubiquitin, ^{K63}polyubiquitin, *etc.*).

Also, as used herein, the nomenclature referring to amino acids of the substrate, from the N-terminus, amino acids of the substrate are abbreviated as ....P3-P2-P1-P1′-P2′-P3′.... The scissile bond is that between the P1 and the P1' residue. The corresponding sites on the enzyme are labeled ...S3-S2-S1-S1′-S2′-S3′ *etc.* Other abbreviations are: UBP, ubiquitin-specific processing protease; UCH, ubiquitin C-terminal hydrolase; and SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

An ε-linked ubiquitin dimer missing the C-terminal glycylglycine (Nε-ubiquitinyl-^{K48}Ub$^{1-74}$) was synthesized as described above for Nε-ubiquitinyl-^{K48}Ub, except that 6 mg/ml of des-glygly-ubiquitin was reacted with 2 mg/ml of native ubiquitin. The reaction was incubated at 37°C overnight. Progress of the synthesis was assayed with HPLC, and terminated by the method outlined above. Under these conditions, polyubiquitin chains are <97% terminated with des-glygly-Ub. The reaction products were separated on Mono S FPLC (Pharmacia).

Nε-Ubiquitinyl-L-lysine and Nε-ubiquitinyl- ^{K48}Ub-L-lysine derivatives were synthesized as above except that the reactions included 200 mM to 500 mM concentration of the particular lysine derivative: either Nα-acetyl-L-lysine (500 mM), Nε-acetyl-L-lysine (500 mM), L-lysine (200 mM), or Nα-acetyl-L-lysine-N-methyl amide (200 mM). These reactions were allowed to incubate overnight at 37°C to assure maximal lysine conjugation. C-8 Reverse phase HPLC was used to monitor these reactions, and the reactions were terminated as described above.

Hydrolysis studies

Hydrolysis rates were measured by incubating the above substrates with homogeneous UCH-L1 or L3. Conditions for assay were essentially as described previously (Wilkinson *et al.*, 1986). Incubation of UCH was performed at 37°C in 50 mM Tris-Cl, pH 7.6, with 5 mM DTT and 50 μg/ml ovalbumin, for varying amounts of time. Substrate concentrations were 15 μM, approximately 20-fold higher than the K_m for ubiquitin ethyl ester. Values are reported as the mean and the standard error of the mean for between 6 and 30 determinations. In cases where no catalysis was observed, the substrate was raised to its highest possible concentration.

Results

P1' specificity

Removal of a single amino acid or small peptide from the C-terminus of ubiquitin must occur during processing of ubiquitin precursors and metabolites (see Table 4). As ubiquitin ethyl ester (Wilkinson *et al.*, 1986) and Ub-DTT (Rose and Warms, 1983) are both rapidly hydrolyzed by UCH isozymes, it was of interest to determine if these enzymes exerted any specificity for residues at the P1′ position of ubiquitin fusion proteins[2]. Such specificity might manifest itself in differential rates of cleavage of α–linked amino acid extensions. FIG. 1 shows the hydrolysis rates obtained with UCH-L1 and -L3 isozymes for Ub-pro, Ub-lys, Ub-his, and Ub-asp,

relative to ubiquitin ethyl ester, the inventors' generic reference substrate. The data show that neither UCH isozyme exhibited a strong preference for the P1′ residue *(1)* immediately following ubiquitin, except when it was proline. Ub-amino acid extensions were hydrolyzed by both UCH isozymes at rates only 1 to 2 orders of magnitude more slowly than UbOEt, whereas Ub-Pro was hydrolyzed at about 3 or 5 orders of magnitude more slowly than UbOEt (Table 3). These rates were determined at 15 μM substrates and probably represent Vmax values. Thus, these UCH isozymes are not selective with respect to the charge or size of residues at the P1′ position when the ubiquitin extension is a single non-proline amino acid residue.

Table 3: Rates of hydrolysis of ubiquitin derivatives by UCH-L1 and UCH-L3

	Substrate	UCH-L1 Activity (μmoles/min/mg)		UCH-L3 Activity (μmoles/min/mg)	
		[1]Mean	[1]SEM	[1]Mean	[1]SEM
1.	Ub-[OEt]	30	6.0	110	22
2.	Nα-Ub-[L-histidine]	6.0	0.9	26	2.6
3.	Nα-Ub-[L-lysine]	7.2	0.7	20	4.0
4.	Nε-Ub-[L-lysine]	3.7	1.4	23	4.0
5.	Nε-Ub-[Nα-acetyl-L-lysine]	6.3	1.3	13	2.6
6.	Nα-(Ub-^{K48}Ub)-[L-lysine]	4.7	0.3	9.9	2.2
7.	Nε-(Ub-^{K48}Ub)-[Nα-acetyl-L-lysine]	5.0	1.0	10	2.0
8.	Nα-Ub-[L-aspartate]	9.9×10^{-1}	9.0×10^{-1}	15	2.4
9.	Nα-Ub-[MQIFVRPR]	1.5×10^{-1}	6.3×10^{-2}	79	8.8
10.	Nα-Ub-[MQIFVKTLTG]	6.0×10^{-3}	1.9×10^{-3}	8.8	1.7
11.	Nα-Ub-[IIEPSLRQLA]	1.4×10^{-4}	6.6×10^{-5}	8.5	0.9
12.	Nα-Ub-[CEP52]	2.1×10^{-4}	4.8×10^{-4}	8.4	5.9
13.	Nα-Ub-[L-proline]	3.9×10^{-4}	2.5×10^{-3}	1.3×10^{-1}	8.8×10^{-2}
14.	Nα-Ub-[UB]	$<1 \times 10^{-5}$	-	$<1 \times 10^{-5}$	-
15.	Nα-Ub-[PRSLDSC]	$<1 \times 10^{-5}$	-	$<1 \times 10^{-5}$	-

Rates of hydrolysis of the indicated substrates are shown. The leaving group is bracketed. The detection limit in this assay is about 1×10^{-5} μmoles/min/mg. [1]The mean and the standard error of the mean were derived from between 6 and 30 replicate measurements.

Table 4: Illustrative C-terminal Extensions of the Proubiquitin Gene Product in Various Organisms

Extension	Organism (# Ub repeats)
-AF	*Acetabularia cliftonii (9)*
-C	*Bos taurus (4), Homo sapiens (3)*

Extension	Organism (# Ub repeats)
-DI	*Caenorhabditis elegans (11)*
-DF	*Petroselinum crispum (6)*
-F	*Geodia cyndonium (6), Nicotiniana sylvestris (6), Pisum sativum (5), Arabidopsis thaliana (5), Glycine max (4), Antirhinium majus (>3), Sus scrofa (>3), Candida albicans (3), Euplotes eurystomus (3),*
-IQA	*Drosophila melanogaster (3)*
-K	*Hordeum vulgare (>2)*
-L	*Dictyostelium discoideum (5 and 3), Trypanosoma brucei brucei (1)*
-M	*Aglaothamnion neglectum (6)*

Table 4 (continued)

-N	*Dictyostelium discoideum (7 and 5), Gallus gallus (3), Phytophtora infestans (3)*
-Q	*Strongylocentrotus purpuratus (10), Zea mays (7), Oryza sativa (6), Tetrahymena pyriformis (5), Avena fatua (4), Neurospora crassa (4)*
-TQTSGKTFMTELTL	*Artemius nauplius (>2)*
-VYASPIF	*Cavia porcellus (4)*
-V	*Homo sapiens (9)*
-Y	*Cricetulus griseus (5), Gallus gallus (4), Mus musculus (4)*

The proubiquitin genes of most organisms encode head-to-tail repeats of the ubiquitin coding sequence with an additional amino acid or peptide at the C-terminus. A wide variety of residues must be cleaved from the polyubiquitin gene containing a variable number of ubiquitin repeats. Hence, the activity responsible for this cleavage are expected to show little P1′ specificity. Note the absence of proline at the junction.

Comparison of peptidase and isopeptidase activities

Because ubiquitin is also conjugated to proteins through an isopeptide bond (*i.e.* through the ε-amino group of lysine), it was of interest to examine whether UCH isozymes could cleave Ub-ε-amino lysine derivatives. It has been shown that UCH-L3 can hydrolyze both types of bonds (Pickart and Rose, 1985), although absolute rates were not determined. As a model isopeptidase substrate, the inventors synthesized Nε-ubiquitinyl lysine by incubation of ubiquitin and lysine with the E1 activating enzyme, the E2-25K conjugating enzyme and ATP. In this synthesis, the excess lysine nucleophile captures the thiolesterified ubiquitin from the transient E2-Ub intermediate, forming exclusively the Nε-ubiquitinyl lysine product and halting further synthesis of polyubiquitin by E2-25k. Both UCH isozymes rapidly hydrolyzed Nε-linked lysine (FIG. 10). Additionally, the rates were essentially identical to those obtained with Nα-linked lysine (Table 2).

Table 2. Refinement Statistics

Resolution Range (Å)	6.0 - 1.8
High resolution shell (Å)	(1.88-1.80)
Rvalue (%)[a]	23.0
	(36.9)
Rfree (%)[b]	28.6
	(36.4)
rmsd(bonds) (Å)[c]	0.010
rmsd(angles) (°)[c]	1.867
#Residues included (total)	205 (230)
#Atoms with occupancy - 0.0[d]	10
#Water molecules	121
<B> ($Å^2$) Protein/Water	27.9 / 36.6
# ϕ/φ angles (%): Most Favored[d]	92.8
Additional	6.7
Generous	0.6
Forbidden	0

[a] Rvalue = 100 * Σ(| |Fp(obs)| - |Fp(calc)| | = Σ|Fp(obs)|

[b] Rfree = Rvalue for a randomly selected subset (5%) of the data that were not used for minimization of the crystallographic residual (Brünger, 1992a).

[c] Stereochemistry was analyzed with PROCHECK (Laskowski *et al.*, 1993).

[d] Non-hydrogen atoms only. Atoms of the Arg-145 and Glu-203 side chains were assigned an occupancy of zero because they lack defined electron density.

A more relevant ε-linked substrate might be an Nε-ubiquitinated peptide similar to the degradation remnants expected to be generated by the action of the proteasome on ubiquitinated proteins. To more closely mimic a peptide bond at the α amino group of an Nε-linked lysine, the inventors synthesized and tested Nε-ubiquitinyl-(N-a-acetyl)lysine as a substrate. The addition of an acetyl functionality to the α-amino group did not affect the hydrolysis rate of Nε-ubiquitinyl-lysine

(FIG. 10). Both isozymes cleaved acetylated and unacetylated substrates at a rate roughly 8 to 10 fold slower than the rate of cleavage of ubiquitin ethyl ester. Subsequent studies showed that there was also no effect of amidating the carboxyl group of lysine with N-methyl amine.

Polyubiquitin processing

Because Nε-ubiquitinyl-lysine was a good UCH substrate, the inventors sought to determine if an Nε-diubiquitinyl lysine derivatives were good UCH substrate. If these enzymes function in the removal of a K48-linked remnant peptide from polyubiquitin, they should process lysine derivatives at the C-terminus of polyubiquitin chains. As a model substrate, the inventors synthesized Nε-(Ub-^{K48}Ub)-lysine and Nε-(Ub-^{K48}Ub)-(N-α-acetyl)lysine. The lysine is removed from these polyubiquitin derivatives at rates identical to the simpler Nε-Ub-lysine derivatives, regardless of the presence of a second ubiquitin (FIG. 1). Neither UCH is able to hydrolyze the K48 isopeptide bond. Neither Ub-^{K48}Ub, nor Ub-^{K48}Ub(des-glygly) is cleaved, even at a four-fold molar excess of enzyme for two hours at 37°C. This hydrolysis rate is therefore more than eight orders of magnitude slower than the ubiquitin esterase rate of either enzyme. This suggests that the ubiquitin binding site on UCH isozymes recognizes a face of ubiquitin distant from the K48 linkage site, and suggests that UCH could function in generating a free C-terminus on polyubiquitin chains by the removal of small peptides and/or cellular nucleophiles.

Fusion peptide processing

It has been postulated that Ubiquitin C-terminal hydrolases could participate in the processing of ubiquitin gene products. It is unlikely that a protein as small as UCH could exhibit specificity for ubiquitin and also a significant portion of the C-terminal extension. Thus, if UCH activity were responsible for processing ubiquitin gene products, then these enzymes would be expected to exhibit specificity for the peptide sequences at the junction between ubiquitin and the C-terminal extension. Model substrates synthesized to test this hypothesis consisted of ubiquitin

followed by the first ten amino acids of the C-terminal extensions; *i.e.*, Ub-CEP52$^{1-10}$ (substrate 11, FIG. 10) and Ub-Ub$^{1-10}$ (substrate 10, FIG. 10).

FIG. 10 shows that isozyme L3 exhibited little selectivity for any of the peptide extensions, cleaving them nearly as rapidly as it cleaves single amino acid extensions. This is also consistent with data which suggests that UCH-L3 has no difficulty cleaving a wide variety of peptide substrates from ubiquitin if the peptides are less than about twenty residues. Interestingly, UCH-L1 exhibited considerably more specificity, showing rates of hydrolysis of these substrates that are over two orders of magnitude slower than the rates of L3-catalyzed hydrolysis (Table 3). Still, UCH-L1 exhibits notable selectivity; the Ub-Ub$^{1-10}$ substrate is hydrolyzed over forty fold faster than the Ub-CEP$^{1-10}$ substrates by this enzyme.

Aside from the natural peptide sequences at the C-terminus of ubiquitin, one other substrate was created. Ub-PRSLDSC, a ubiquitin-peptide fusion with proline at the P′ cleavage junction was created by a PCR™ error that resulted in the read-through of the reading frame into the vector multicloning site. Neither enzyme was able to cleave this fusion peptide at a measurable rate, in spite of the fact that UCH-L3 is able to cleave Ub-pro. The hydrolysis rate of these peptide fusions was more than seven orders of magnitude slower than that for UbOEt.

Ubiquitin proprotein processing

Because model substrates containing the first ten residues of ubiquitin proproteins were hydrolyzed by UCH isozymes, the inventors determined the rate of cleavage of full-length ubiquitin gene products by these enzymes. Purified α-linked Ub oligomers were very slow substrates for UCH-L1, and were not cleaved at all by UCH-L3 (FIG. 10). Micromolar UCH-L1 was able to cleave Nα-diubiquitin at 37°C *in vitro* with a half-life of thirty minutes. This corresponds to a rate of at least 6 orders of magnitude slower than for UbOEt. UCH-L1 is reported to exist at 1-2 % of total soluble brain protein (Day, 1990).

The zinC-finger fusion proteins UbCEP52 and UbCEP80 are the two other natural ubiquitin proprotein substrates studied. High amounts (100 mU) of either recombinant UCH added to bacterial expression lysates for two hours failed to hydrolyze UbCEP52 or UbCEP80 to their monomeric components, based on immunoblotting of the expression lysates. Because UbCEP52 was more highly expressed than UbCEP80, and because the antibodies to CEP52 had a higher titer and were more specific than the anti-CEP80 antibodies, the UCH-catalyzed hydrolysis of the UbCEP52 protein was further characterized.

Surprisingly, purified UbCEP52 was hydrolyzed by both enzymes, though the L3 isozyme catalyzed the reaction much more rapidly (FIG. 10 and FIG. 11). The rate of processing of UbCEP52 by UCH-L3 approaches the rate of hydrolysis of the Ub-amino acid extensions, about 200 min^{-1}. To confirm the specificity of this reaction, SDS-PAGE and immunoblotting were used to identify the products (FIG. 11. The appearance of ubiquitin and CEP52 detected by SDS-PAGE is consistent with the rates measured by HPLC. UCH-L1 also hydrolyzed the substrate to a measurable degree, but the rate was 2.1×10^{-4} μmoles/min/mg, or about 10^{-5} the rate of ester hydrolysis.

The above results suggest that the bacterial lysates contain something which interferes with UbCEP52 hydrolysis, but not with UbOEt hydrolysis. UbCEP52 possesses a C_2H_2 zinC-finger binding motif, so it was determined whether binding of zinc could inhibit the UCH-L3 hydrolysis of UbCEP52. $Zn(OAc)_2$ (10 mM) did not inhibit UbCEP52 hydrolysis by either enzyme. Whether the zinC-finger motif binds metal *in vivo* remains to be elucidated, however, addition of excess metal ion does not inhibit the processing of the proprotein by UCH.

The presence of a zinc finger motif in a ribosomal protein is presumptive evidence of nucleic acid binding. To test if binding of nucleic acid inhibited processing, assays were performed in the presence of nucleic acids. *In vitro* addition

of 50 μg/ml of either plasmid DNA, or a double stranded 26-base pair DNA cassette inhibited the hydrolysis of UbCEP52 by 50%, whereas a single stranded 42-base pair oligodeoxynucleotide at the same concentration was only minimally effective (FIG. 12). Whole yeast RNA was even better at inhibiting processing, showing 60 to 80% inhibition. Phenol/chloroform extraction of this RNA did not improve the processing, suggesting that the inhibition was not due to other contaminating proteins in the RNA preparation. Also, preincubation of the RNA with RNAseA restored the UbCEP hydrolysis rate back to control rates. These results imply that the nascent proprotein can only be cleaved by UCH before nucleic acids are bound to the fusion peptide, and that assembly into the ribosomal subunit would probably prevent processing.

UCH isozymes can co-translationally process ubiquitin proproteins.

Ubiquitin proproteins are very rapidly processed *in vivo* (Finley *et al.*, 1989; Baker *et al.*, 1992). The UCH isozymes appear to be very efficient at processing peptides from the C-terminus of ubiquitin, but not if the C-terminal extension has a chance to fold into a tight, globular domain (see above). Further, only UCH-L1 is able to rapidly process the proubiquitin precursor, and this isozyme is present at low levels in most tissues. These observations suggest that processing of some ubiquitin gene products may occur before folding or subunit assembly is completed. To test the idea that UCH isozymes can process UbCEPs co-translationally, the inventors co-transformed cells with vectors expressing UCH and Ub proproteins in various combinations. UCH-L1 was found to hydrolyze polyubiquitin (60%) and UbCEP80 (50%), but not the UbCEP52 (>5%) (FIG. 13). This data is consistent with the above data from peptide hydrolysis, in that UCH-L1 prefers to hydrolyze ubiquitin-like peptides and also hydrolyzes the complete proubiquitin, albeit slowly. In contrast, UCH-L3 was found to hydrolyze both Ub-CEP fusions, but not proubiquitin (FIG. 13).

The present invention describes attributes relating to the substrate specificity of two closely related UCH isozymes, UCH-L1 and -L3. The hydrolysis rates reported herein were determined at 15 μM substrates, approximately the same concentration as that of total ubiquitin in the cell. The Km for hydrolysis of ubiquitin ethyl ester is approximately 1 μM and is identical to the ubiquitin binding constant (Larsen *et al.*, 1996). Thus, in the absence of unfavorable interactions between the enzyme and the leaving groups, the measured rates would reflect Vmax values. With some of the poorer substrates however, the slower observed rates of hydrolysis may be due to higher Km values for these substrates. Irrespective of the reasons for the slower rates of hydrolysis, it is clear that these differences are manifest at concentrations that are many times that observed in a cell and that the rates reported may overestimate the relative rates of hydrolysis that would pertain *in vivo*.

Ubiquitin binding to the S site(s)

The available evidence suggests that the S sites form an extensive binding site for intact ubiquitin. The only demonstrated activity of UCH isozymes is for cleavage of amide and ester bonds at the C-terminus of ubiquitin. There is little or no affinity for small peptides at the C-terminus of ubiquitin (such as glycylglycine) but ubiquitin is bound with a micromolar binding constant (Larsen *et al.*, 1996). Ubiquitin aldehyde is a tightly-bound inhibitor of these enzymes. Further, NMR measurements have confirmed an extensive area of contact between ubiquitin and UCH-L3; encompassing over 20% of the surface residues on ubiquitin (Wand and Wilkinson) including the C-terminus. This contact surface cannot include the N-terminus of ubiquitin, as a hexahistidine tag at the N-terminus has little or no effect on the rates of hydrolysis. In agreement with this result, it has been shown that these enzymes bind to immobilized $(his)_6$ ubiquitin (Beers and Callis, 1993). The surface of ubiquitin containing K48 is also not in the S1 recognition site on ubiquitin, as Nε-Ub-^{K48}Ub derivatives are good substrates for cleavage of the leaving group from the free C-terminus (FIG. 10 and Table 3). Nε-Ub-^{K48}Ub does not appear to be a substrate, probably because the leaving group ubiquitin is tightly folded against the C-terminal

face of the distal ubiquitin. Finally, the interactions between ubiquitin and UCH-L3 are predominantly ionic, as evidenced by the previously observed inhibition of binding and activity by salt (Larsen *et al.*, 1996).

S1′ Specificity

Many different amino acids and peptides are found as natural extensions of ubiquitin genes in eukaryotes (Table 4). Putative processing enzymes would have to either have broad specificity at the P1′ site or exhibit significant sequence variability from species to species in order to accommodate their respective species-specific leaving groups. In fact, UCH sequences are very similar across species, with rat, human and bovine UCH-L1 being over 98% identical. The inventors' results show that UCH isozymes exhibit very little specificity for the P1′ residue of ubiquitin substrates (FIG. 10) with essentially identical rates with acidic, basic or neutral leaving groups. If UCH isozymes were responsible for processing the amino acid extensions of ubiquitin gene products, they would exert little selective pressure on the nature of that leaving group. This may be why there seems to be little selective pressure to maintain the identity of this extension amino acid (see Table 4).

As both α-, and ε-linked derivatives have to be processed from the C-terminus of ubiquitin, the selectivity for cleavage of these two types of amide bonds was examined. These enzymes exhibited little or no discrimination based on the identity of the amide bond to lysine (α vs. ε), the charge at the other amine (free amine vs. N-acetyl), or the charge at the carboxyl group (carboxyl vs. N-methyl amide). Further, the same derivatives can be efficiently processed from the C-terminus of the polyubiquitin chain. Thus, at least with small leaving groups, these enzymes could be involved in processing both the amino acid and small peptide extensions of various gene products, as well as the Nε-(poly)ubiquitinyl lysine expected to be generated by the action of the proteasome on polyubiquitinated protein substrates.

The S site(s) Will Not Bind Larger Protein Domains

FIG. 10 demonstrates that UCH-L3 is generally able to hydrolyze a variety of small peptide fusions at the C-terminus of ubiquitin. To examine if there was any selectivity based upon P′ sequences, the inventors have also measured the rates of processing of the ubiquitin gene products and short model substrates consisting of ubiquitin fused to the first ten amino acids of the C-terminal domains.

UCH isozymes exhibit significant selectivity in the processing of the ubiquitin gene products. UCH-L3 is able to efficiently process the Ub-CEP52 gene product, but not the Ub-CEP80 or proubiquitin gene products. Isozyme L1 is only able to slowly process the proubiquitin gene product *in vitro* and *in vivo*. It has been reported that the yeast homolog, YUH1, also exhibits a similar selectivity in that small fusion proteins can be efficiently processed, but not larger fusions (Miller *et al.*, 1989). The drosophila homolog has been reported to be able to process α-ubiquitinyl-IκBα (314 amino acids), but not larger fusions (Roff *et al.*, 1996).

Interestingly, nucleic acid binding to Ub-CEP52 likely prevented its processing by UCH-L3 (FIG. 12). The addition of nucleic acid to UbOEt had no effect on its hydrolysis, suggesting that the nucleic acid was directly binding to Ub-CEP52 and causing a conformational change which prevented processing. The binding of nucleic acid by Ub-CEP52 is not unexpected; the CEP domain contains a zinC-finger motif, the protein is a ribosomal subunit, and mutants in this gene are defective in rRNA processing.

The above results suggest that the selectivity of the S′ sites may be based on factors other than size. One factor could be the accessibility of the peptide bond at the C-terminus of ubiquitin. It might be expected that ubiquitin fusion proteins with significant mobility and flexibility at the junction could be good substrates while those that are more constrained (proline) and/or sterically restricted (large) would be poor substrates. This is consistent with the ligand-induced inhibition described above

(*i.e.* binding of nucleic acid may cause a less mobile conformation around the Ub-CEP52 junction) as well as the restricted nature of the substrate binding cleft observed in the UCH-L3 crystal structure (Johnston *et al.*, 1997).

Substrate Specificity Based on the P′ Peptide Sequence

An alternative explanation for the observed selectivity in processing of ubiquitin gene products is that the enzymes may exhibit significant selectivity based on the amino acid sequences binding to the S′ sites. To examine the contribution of the P′ residues to the observed selectivity, the inventors have used model substrates consisting of ubiquitin fused to small peptides, including the first ten amino acids of each ubiquitin gene product. FIG. 10 demonstrates that UCH-L3 is not very selective for the P′ residues, processing every small peptide tested except those containing proline at the scissile bond. This specificity is similar to that reported for the yeast UCH; *i.e.* ubiquitin extended by E, C, D, G, T, or M (but not P) was hydrolyzed efficiently (Miller *et al.*, 1989). This may be because the secondary amine of the proline has a somewhat higher pK_a than the primary amino group in the peptide bond of most amino acids, or it may reflect a steric constraint imposed at the scissile bond. UCH-L3 is unable to process at proline in the Ub-PRSLDSC peptide fusion. It is likely that the presence of proline at the P1′ position "kinks" the peptide such that it can not be accommodated in the active site cleft. The presence of a proline at position P4′ (Ub-CEP52$^{1-10}$, substrate 11) or P7′ (Ub-Ub$^{1-5}$-RPR, substrate 9) has little effect on the rate of peptide processing by UCH-L3, suggesting that the cleft may be considerably less restricted at that distance from the active site nucleophile. The Ub-Ub$^{1-10}$ construct is processed very effectively by UCH-L3, but the Ub-Ub fusion protein is not cleaved at all, reinforcing the conclusion that a tightly folded domain at the C-terminus of ubiquitin is not generally a substrate for these enzymes.

In contrast to the permissiveness of UCH-L3 processing, the processing by UCH-L1 is more selective, with ubiquitin related peptide fusions being reasonable substrates and Ub-CEP52$^{1-10}$ being a poor substrate. While it is not clear whether this

selectivity is due to subsite specificity at P1′-P3′, or the presence of proline in sites P4′-P7′, it is clear that this is a much more selective enzyme. This specificity may be related to interactions with an occluding loop which is postulated to form part of the S′ sites on the UCH family of enzymes.

Co-translational Processing

These results demonstrate that there is considerable selectivity in the processing of ubiquitin gene products by these UCH isozymes. UCH-L3 appears to prefer processing of Ub-CEP gene products, while UCH-L1 is very selective for the proubiquitin gene product. There is, however, some question as to the physiological significance of these processing events, especially those catalyzed by UCH-L1 which occur at an extremely slow rate. This led the inventors to ask if these enzymes might be involved in co-translational processing. Normal processing is known to be extremely efficient, with no evidence for accumulation of intermediates in the process. Further, if these enzymes are involved in processing, they must act before significant assembly into ribosomal subunits, and/or folding of stable domains C-terminal to ubiquitin. When enzyme and substrate were co-expressed in *E. coli*, the efficiency of processing was high and the selectivity was similar to that observed above. UCH-L1 was able to process over 80% of the proubiquitin gene product, and little of the Ub-CEP gene products, while UCH-L3 was most efficient in processing the Ub-CEP fusion proteins (> 50% processed). Thus, it appears that processing is much more efficient if the enzyme is present during the synthesis of the substrate. Confirmation of this phenomenon was attempted by demonstrating the association of UCH-L3 with polyribosomes synthesizing the substrates. When an *in vitro* transcription/translation system is supplemented with DNA encoding the substrate, endogenous UCH activity is found exclusively in the soluble fractions. Even upon addition of exogenous UCH isozymes, little or no UCH activity can be found stably associated with the ribosomes. It may be that the association is only fleeting and unstable, or it may be that processing occurs after release of the substrate polyprotein from the ribosome but before folding of the complete protein.

Molecular Basis of Specificity

As shown above, the x-ray crystal structure of UCH-L3 has a core catalytic structure that strongly resembles cathepsin B, a papain-like protease. The active site groove is occluded by two loops, and it is postulated that a substrate-induced conformational change is required to clear the cleft and allow access to the active-site cysteine. Thus, only ubiquitin derivatives are substrates because only they can form the extensive interactions with the S′ site required to trigger the necessary conformational change generating the active conformation of the enzyme.

Specificity for P′ residues must be determined by the residues lining the corresponding S′ sites on the UCH enzymes. The sequence of these proteins varies widely in several areas, including a region just N-terminal to the active site histidine. This sequence is disordered in the UCH-L3 structure, but may be positioned to form a significant contact region with the P′ residues of substrates (Johnston *et al.*, 1997). Thus, it is likely that this hypervariable region is important in determining substrate selectivity and the somewhat shorter loop near the active site cysteine in UCH-L1 restricts the possible substrates by conferring a narrower or more restricted active site cleft. These predictions could be tested by obtaining the structure of UCH-L1 and/or using site directed mutagenesis and domain swapping approaches.

Potential Physiological Roles for UCH Isozymes

The possible physiological roles for UCH isozymes are limited by the temporal and spatial patterns of expression of the enzymes and putative substrates, as well as by restrictions imposed by the substrate specificity examined here. With respect to the former, there is a marked tissue specificity to the expression of UCH isozymes, with UCH-L1 being expressed at very high levels in neural and diffuse neuroendocrine tissues, and UCH-L3 being expressed primarily in hematopoetic tissues (Wilkinson *et al.*, 1992). There is little evidence of temporal regulation, as these enzymes seem to be present in all stages of the cell cycle and both early and late

in development. A third isozyme, UCH-L2 has been reported to be widely distributed, albeit at lower levels than either of the two isozymes studied here (Wilkinson *et al.*, 1992).

The distribution of putative substrates is more difficult to assess, although the results discussed above suggest that substrates will include the ubiquitin proproteins and small molecule adducts of ubiquitin. The latter are expected to be widely distributed, as there is extensive activation and conjugation of ubiquitin in all tissues examined. All of the intermediates in the enzymatic activation of the C-terminus of ubiquitin are thiol esters and they are effectively trapped by reaction with small molecular weight thiols and amines. There is a much more specific expression of ubiquitin pro-proteins. Rapidly growing cells have been shown to express high levels of ubiquitin-ribosomal fusion proteins, while more differentiated cells (such as neurons), express ubiquitin primarily from the proubiquitin locus.

These considerations suggest that UCH-L1, the neuronal specific isozyme, may be more efficient at cleaving the proubiquitin precursor, while the hematopoetic specific UCH-L3 might prefer ubiquitin ribosomal fusion proteins as substrates. These predictions are borne out using ubiquitin fusion peptides as substrates. UCH-L1 is found at high levels only in neurons and diffuse neuroendocrine tissues, and it cleaves the proubiquitin model substrate (Ub-Ub^{1-10}, substrate 10) much faster than it cleaves the ubiquitin ribosomal fusion protein model substrate Ub-$CEP52^{1-10}$ (substrate 11). UCH-L3 on the other hand can cleave all the model substrates at a significant rate. The specificity of co-translational cleavage of the full length gene products reflects the results with small peptide fusions, implying that a portion of the UCH specificity derives from interactions with P′ residues. Large, tightly folded leaving groups are not substrates for this class of enzyme, although there are differences in the selectivity demonstrated by each enzyme.

These results support the idea that UCH enzymes are responsible for co-translational processing of the polymeric ubiquitin gene products and/or salvage of

ubiquitin from small molecular weight adducts. Only ubiquitin derivatives will be substrates, probably because of the obligatory substrate-induced conformational change required to generate the active enzyme. Isozymic differences may be due to sequence differences in the hypervariable loop region and presumably reflect the metabolic flux of the tissues wherein these isozymes are expressed, although confirmation of this role awaits identification of mutations in these loci or development of transgenic animal models.

EXAMPLE 3 - SUBSTRATE BINDING AND CATALYSIS BY UBIQUITIN C-TERMINAL HYDROLASES

There are several polymeric ubiquitin structures which contribute to the biology of ubiquitin. Ubiquitin is post-translationally conjugated to a variety of proteins present in the cell. Proteins can be multiubiquitinated by the addition of ubiquitin to several surface lysines or polyubiquitinated by the addition of ubiquitin to one surface lysine followed by the addition of another ubiquitin to K48 of the first ubiquitin. Long polymeric chains can thus be assembled by the conjugation of ubiquitin to the distal end of this chain. These polyubiquitinated proteins are then degraded by the 26S proteasome to yield free amino acids and the polyubiquitin chain (Eytan *et al.*, 1989; Hough *et al.*, 1987). The ubiquitin isopeptide bond linking these subunits must be hydrolyzed by the action of specific proteases. This hydrolysis is necessary to salvage ubiquitin for conjugation as well as to prevent the accumulation of free polyubiquitin chains which are known to bind to the 26S proteasome and inhibit proteolysis (Deveraux *et al.*, 1994). The inventors have recently shown that this reaction is catalyzed by a 93 kDa protein termed isopeptidase T (Wilkinson *et al.*, 1995).

In addition to isopeptide-linked polymeric ubiquitin, the cell must also proteolytically process polymeric ubiquitin linked by peptide bonds. Ubiquitin is always translated from mRNA as a fusion protein, either with additional copies of ubiquitin itself or with one of two different zinc fingers (Ozkaynak *et al.*, 1987). The proubiquitin gene product consists of multiple copies of ubiquitin, is induced by

stress, and must be processed to monomeric ubiquitin by the action of a processing protease (Finley *et al.*, 1987. Similarly, two ubiquitin-zinc finger fusion proteins arc synthesized in rapidly growing cells. They must be accurately processed to free ubiquitin and the zinc finger CEP52 arid CEP80, which are ribosomal proteins (Finley *et al.* 1989).

The proteolytic processing of both α- and ∈-amide linked ubiquitin occurs at the carboxyl group of glycine 76, suggesting that such processing proteases might have specificity for binding the ubiquitin monomer. Several proteases with these properties have been described, including those known as ubiquitin C-terminal hydrolases (Pickart and Rose, 1985), ubiquitin specific proteases (Tobias and Varshavsky, 1991; Baker *et al.*, 1992), or isopeptidases (Matsui *et al.*, 1982). These proteases can be grouped into two families. The ubiquitin-specific protease family (UBP) consists of several distantly-related proteases of 50-300 kDa which show several homologies around an active site thiol and a putative active site histidine. (Abbreviations used: CD, circular dichroism; DTT, DL-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; IPTG, isopropyl β-D-thiogalactopyranoside; MES, 2-[*N*-morpholino]ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PCR™, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Ub, ubiquitin; UbOEt, ubiquitin ethyl ester (Wilkinson *et al.*, 1986); UBP, ubiquitin specific proteases (Baker *et al.*, 1992); UCH, ubiquitin carboxyl-terminal hydrolase (Wilkinson *et al.*, 1989). This family is also known as UCH family 2 and includes at least 11 members in yeast with other known homologues in mammals and *Drosophila* (Papa and Hochstrasser, 1993; Wilkinson *et al.*, 1995). They are thought to be involved with processing various ubiquitin-protein fusions expressed in eukaryotic cells and/or the polyubiquitin degradation signal (Tobias and Varshavsky, 1991; Baker *et al.*, 1992, Wilkinson *et al.*, 1995). The ubiquitin carboxyl-terminal hydrolase (UCH) family is a group of small, closely-related thiol proteases consisting of three mammalian isozymes (Wilkinson *et al.*, 1989) and with close homologues in *Saccharomyces cerevisae* (Liu *et al.*, 1989) and *Drosophila melanogaster* (Zhang *et*

al., 1993). They exhibit no apparent homology to the UBP family, and this dissimilarity implies two functionally convergent ancestral genes. The presence of multiple, tissue specific UCH isozymes (Wilkinson *et al.*, 1992) suggests that the metabolism of ubiquitin may also be tissue specific. These enzymes prefer small leaving groups and/or extended peptide chains at the C-terminus of ubiquitin. It is postulated that they are involved in the co-translational processing of the proubiquitin and ubiquitin-zinc finger fusion proteins which are the ubiquitin gene products. It is not clear how any of these processing proteases distinguish among the several types of polymeric ubiquitin or achieve hydrolytic specificity. Since many, if not all of them, bind ubiquitin, their hydrolytic specificities and *in vivo* rates may depend on the specific recognition of leaving group peptides, side chains, or proteins in the non-ubiquitin portion of the substrate the P′ site according to the nomenclature of Schechter and Berger (1967).

The UCH class of proteases is unique in several ways. Firstly, they appear to represent a new family of thiol proteases, as there is no apparent sequence homology to any other proteases. As such, the structure and function of these proteins is of general interest. Secondly, they are extremely specific, cleaving only after the C-terminal glycine of ubiquitin. Recombinant UCH's can be expressed in high amounts in *Escherichia coli,* do not form inclusion bodies, and are nontoxic to the host. This is consistent with the enzymes having a very narrow spectrum of proteolytic specificity. In contrast with members of the papain super-family, which exhibit broad P site specificity (Fox *et al.*, 1995), UCH's show strict and narrow P site specificities for the RGG C-terminus of Ub. Finally, these enzymes are mechanistically unique in that binding of ubiquitin results in a finite equilibrium of thiol ester between the C-terminus and the active site thiol of the protease. Thus, the enzyme- substrate complex (ubiquitin + UCH-L3) can be reduced by borohydride to give the thiohemiacetal of the protease and ubiquitin aldehyde (Pickart and Rose, 1986). The energy required to form even a small amount of intermediate thiol ester must result from extensive binding interactions between ubiquitin and the protease. For these reasons, a more detailed structural analysis of the UCH family is of interest.

The inventors previously reported four UCH activities from bovine thymus with specificity for cleavage of the C-terminal ethyl ester of ubiquitin (Mayer and Wilkinson, 1989). Three of these enzymes are approximately 25 kDa in size, while the fourth activity is of higher molecular weight and is less well understood. These ~25 kDa activities are named UCH-L1, UCH-L3 and UCH-L3 on the basis of their order of elution from a DE-52 anion exchange matrix, and the inventors have found UCH-L1 to be identical to the protein PGP 9.5 (Wilkinson *et al.*. 1989). This hydrolase is most highly expressed in neuronal and neurosecretory tissues. Additionally, it is selectively accumulated (along with ubiquitin conjugates) in the plaques of Alzheimer's disease as well as in lesions of other neurodegenerative diseases (Lowe *et al.*, 1990). In the present work, UCH-L1 was cloned and mutagenized, and three important residues were identified, including the active site cysteine and histidine. Various spectral characterizations demonstrate that UCH contains α/β folding motifs and that the UCH mutants studied demonstrate normal parameters of thermal denaturation. Thus, these residues appear to be unimportant for protein folding or stability. As UCH-L1 is insoluble above 1.5 mg/mL, the physical characteristics of a more tractable isozyme, UCH-L3, were studied. The inventors find that ubiquitin binding to this isozyme is stoichiometric and inhibited by salt. These data provide the first detailed analysis of the binding of ubiquitin with one of its adjunct enzymes, and so provides additional insights into the nature of the ubiquitin UCH protein-protein binding interactions.

PROCEDURES

UCH Cloning and Subcloning. The cDNA encoding UCH-L3 from the plasmid pBHA (Wilkinson *et al.*, 1989) was subcloned into the T7 expression vector pRSET (Invitrogen, San Diego, CA). Plasmids pBHA and pRSET were digested with *Nde*I and *Eco*RI (New England Biolabs, Beverly, MA). The 780 bp UCH-3 insert and the 2810 bp vector were gel-purified and ligated, and the resultant plasmid was used to transform Top 10 F' *E. coli* (Invitrogen). Colony minipreps were screened, and

several which were linearized by *Nde*I to give a 3.5 kb linear fragment were selected. An insert from a positive clone was sequenced to verify the integrity of the plasmid ("pRS-UCHL3") and was used to transform the *E. coli* expression host BL21(DE3) (Novagen). On IPTG induction, cells with this plasmid overexpressed a 25 kDa protein which cross-reacted with anti-human UCH-L3 polyclonal antibodies. Cytosol from the sonicated cells showed significant enzymatic activity in cleaving ubiquitin ethyl ester (Wilkinson *et al.*, 1986). Human UCH-L1 was cloned *via* reverse transcriptase-mediated polymerase chain reaction (RT-PCR™, Perkin-Elmer Cetus) from a human fetal brain poly-A RNA library (obtained from Dr. Stephen T. Warren) using primers to the known human PGP9.5 sequence (Day *et al.*, 1990). It was subcloned by the dideoxy method (Sanger *et al.*, 1977), subcloned into pRSET to give pRSL1, and transformed into BL21 *E. coli* as described above. Sequencing revealed two apparent PCR™ errors affecting the codons for residues 73 and 200. Since the change at codon 200 was silent, it was not corrected. The codon at position 73 was repaired as follows. A rat PGP9.5 (UCH-L1) fragment (Kajimoto *et al.*, 1992) was amplified by PCR™ to generate a new silent 5′ *Bss*HII site. The resulting *Bss*HII/*Dra*III cassette codes for identical residues in the rat and the human sequences and so was inserted into pRSL1 in place of the human gene fragment. The construct was sequenced and shown to have the correct predicted amino acid sequence.

UCH Purification. The inventors cloned, expressed, and purified recombinant UCH-L1 and UCH-L3 to study their physical and enzymatic properties. With the exceptions noted, the purification of all UCH isozymes and mutants was similar. A single colony of BL21(DE3) carrying the pRSET-UCH L3 plasmid was inoculated into 2 L of LB media (Sambrook *et al.*, 1986) and grown at 37°C to an absorbance of 0.8 at 600 nm. IPTG (Sigma, St. Louis, MO) was added at 0.4 mM, and the cells were incubated for an additional 1.5 h before the bacteria were centrifuged at 4000g and the pellets were collected. After induction, UCH levels reached an average of 15% of the soluble *E. coli* protein. The cell paste (16 g) was resuspended in 100 mL of lysis buffer (50 mM Tris-HCl, pH 7.5, 10 mM DTT, 50 μM PMSF, 1 mM EDTA, 10 mM $MgCl_2$). Lysozyme was added to 10 000 units/mL for 30 min, and the

suspension was sonicated (Heat Systems, Inc.). The debris was removed by centrifugation at 10 000g for 40 min. The supernatant was concentrated to 50 mL by ultrafiltration (Amicon, YM-10) and applied to a 200 mL Fast Flow Q-Sepharose column equilibrated with buffer A (50 mM Tris•HCl, pH 7.6; 0.5 mM EDTA; 5 mM DTT). The column was eluted with a 300 mL linear gradient to 0.5 M NaCl in buffer A. Fractions with ubiquitin esterase activity eluted at 265 mM NaCl and contained the 25 kDa protein as determined on SDS-PAGE. Enzymatically active fractions from ion exchange were pooled and concentrated to 30 mL and applied to a 1 L Sephadex G-100 Superfine gel filtration column (Pharmacia) in buffer A. Active fractions were pooled again and shown to be >98% pure by Coomassie-stained SDS-PAGE. These detailed enzymes have been used for kinetic studies and for the CD and UV spectroscopy, but for Raman spectra the enzymes were further purified on Mono Q FPLC anion exchange, using the same buffers and gradient as in the ion exchange step described above. The homogeneous fractions were pooled and concentrated by ultrafiltration. Purifications of UCH-L1 were similar to that for UCH-L3, except that the anion exchange salt gradients were 1-300 mM NaCl, with UCH-L1 eluting at 110 mM. Homogeneous UCH-L1 is obtained in two steps, due to slightly higher expression levels and weaker binding to Q-Sepharose. The inventors find the specific activities of homogeneous recombinant UCH-L1 and UCH-L3 are 30 and 110 μmol/min/mg, respectively, using ubiquitin ethyl ester as the substrate. By comparison, UCH-L1 from bovine brain has a specific activity of 25/μmol/min/ mg, and UCH-L3 purified from calf thymus exhibits a specific activity of approximately half the recombinant value. These enzymes are therefore fully active and has been shown to bind one mole of substrate per mole of enzyme (see below), suggesting that they are fully functional as purified.

Mutagenesis. Mutagenesis of UCH-L1 was performed using a combination of M13-based (Kunkel, 1985), cassette subcloning, and PCR™ methods. In M13 mutagenesis, UCH-L1 was excised from pRSET with *Xba*I *and Hin*DIII (New England Biolabs, Beverly, MA) and inserted into M13mp18 at the same sites. Annealing, T7 polymerase extension (T7 Sequenase, USB), and ligation of primers

(containing a new, silent *Hpa*I site 5′ to the mutation) with purified uracil-containing single-stranded M13 DNA generated the UCH-L1 H161D and H161Y mutants. These mutants were identified by screening plaque minipreps (Sambrook *et al.*, 1986) for susceptibility to *Hpa*I digestion. The new *Hpa*I site was then used to create the mutations H161Q, H161N, and H161K: degenerate cassettes produced by PCR™ were inserted by their *Hpa*I *and Kpn*I sites into pRSL1 and sequenced. Lastly, C90S and D176N mutant PCR™ cassettes were made and inserted into the *Bss*HII and *Dra*III (C90S) or *Bss*HII and *Bsm*I sites (D176N). In all cases, the cassettes were always smaller than 400 base pairs and were sequenced after insertion into the expression vector to verify the absence of Taq poly-merase-induced mutations. All isozymes and mutants were expressed in BL21(DE3) cells, and the supernatants from lysozyme lysis were assayed. In most cases, the mutants were purified as above and their catalytic velocities and Michaelis constants were determined (Wilkinson *et al.*, 1986).

UV-Vis, CD, and Raman Spectroscopy. UV-visible spectra from 190 to 800 nm were acquired on a CARY 219 dual-beam spectrophotometer. CD spectra were obtained on an Aviv Associates 62DS, using 10 or 1 mm path length quartz cuvettes (Hellma, Forest Hills, NY) at 25.0 ± 0.1°C. Each spectrum was the average of five scan repetitions. CD spectra of the native protein were collected at 0.95 mg of protein/mL (40 μM) with 1 or 10 mm path length cells. To monitor ubiquitin binding by CD spectroscopy, ubiquitin and UCH-L3 (1 mL, 4 μM) were placed in separate compartments of a dual-compartment 9 mm cell, and the spectrum was recorded. The contents of the compartments were then mixed, and the spectrum was again recorded. The former spectrum was subtracted from the latter to give the difference binding spectrum. A similar procedure was used for determining the effects of ubiquitin binding on the UV absorbance spectra, but with UCH-L3 and ubiquitin at 20 μM.

Circular dichroic spectroscopy was used to monitor the thermal denaturation of UCH at protein concentrations of 0.1 g/L (4 μM) in 1 mm path length cells, or at 0. 1 g/L in 10 mm path length cells. The latter conditions were used for the H161K and

H161Y mutants, which aggregated at higher concentrations. The temperature was controlled with a Hewlett Packard 89100A temperature controller equipped with an immersible temperature probe. The temperature scan rate was varied over a 4-fold range to confirm that measure-merits were made at equilibrium. Scans in both directions (heating and cooling) confirmed that the transitions measured were reversible. The fraction of native protein present at each temperature was calculated assuming a two-state transition between the initial and final spectra obtained, *i.e.*, at any temperature the fraction of native species = (final ellipticity - observed ellipticity)/(final ellipticity - initial ellipticity). Thermodynamic parameters were calculated from plots of ln K_{eq} vs $1/T$ or by curve fitting in Sigma Plot 4.16 for Macintosh. Equilibrium constants were used to calculate thermodynamic state functions according to K_{eq} = min + ((max - min)/(1 + 1/exp(s - h/x))) where x = T, s = ∂H/8.314 J/K mol and $h = \partial H$/8.314 J/mol.

Nonresonance Raman spectra were recorded using the 488 nm emission line of an argon laser (Spectra Physics model 165). Light scattered from the sample at 90° to the incident laser beam was dispersed by a holographic diffraction grating in a 0.6 m triple monochromator (Triplemate, Spex Industries, Metuchen, NJ) and detected by an intensified photo-diode array detector (Princeton Instruments, Trenton, NJ). Power at the sample was less than 100 mW. The known Raman lines of toluene calibrated the system for each measurement, making the measured frequencies accurate to ± 1 cm^{-1}.

Equilibrium Gel Filtration. Equilibrium gel filtration measurements were performed as described (Hummel and Dreyer, 1962) with the following modifications. Tandem Superose 6 and 12 columns (0.5 × 30 cm, Pharmacia) were equilibrated with running buffer (30 mM Tris•HCl, pH 7.5; 5 mM DTT) containing 50 µg of ubiquitin/mL. After equilibration with three column volumes, the ubiquitin concentration in the effluent was identical to that in the applied buffer. Purified UCH-L3 (100 µL, 5.8 µM) was supplemented with ubiquitin to a final concentration of 50 µg/mL (5.8 µM) and applied to this column. The concentration of ligand (ubiquitin) in the effluent was determined in triplicate by HPLC using a Waters WISP

710 B autoinjector and a Gilson HPLC equipped with a Spectra Physics SP4290 integrator (Wilkinson *et al.*, 1986). To determine the effect of salt on ubiquitin binding, the studies were repeated in the presence of 0.5 M NaCl.

RESULTS AND DISCUSSION

UCH Isozyme Family. Ubiquitin C-terminal hydrolases comprise a small, newly defined, and novel family of thiol proteases. Among these, UCH-L3 is the best-characterized member. Human (Wilkinson *et al.*, 1989) *Drosophila* (Zhang *et al.*, 1993), and yeast (Liu *et al.*, 1989) homologues have been described. These known UCH sequences are aligned in FIG. 14, where only residues found in at least three sequences are highlighted. All of these enzymes have slightly acidic isoelectric points (p*I* ~ 5.0) and molecular weights between 24 and 27 kDa. The numbering system used here corresponds to the human UCH-L1 residues. A number of areas in the sequence show a high degree of identity, most notably at positions 88-102 (the amino acid numbering system refers to the UCH-L1 sequence) (containing a conserved cysteine), 109-118, and 161-178 (containing a conserved histidine and an ELDGR sequence. Many of the positions in the aligned sequences are identical in all four sequences (44/249) or are similar in all four (52/249).

This degree of similarity in primary sequence and physical properties is usually taken as evidence of similar secondary and tertiary structure. In support of this assumption, all four UCH sequences give essentially identical plots of Kyte-Doolittle hydropathy. This suggests that the structural properties of these isozymes may be similar. The high homology also implies that the differential enzymatic specificity of each is a consequence of a few sequence differences at the substrate recognition site. A basal collection of UCH residues is probably necessary for proper folding and ubiquitin binding. These binding residues are expected to be on the surface of the protein and in regions that show significant sequence homology in the alignments shown in FIG. 14. Additionally, catalytic residues are expected to be near the surface but are generally at the bottom of a cleft or invagination of the protein

surface. To examine these relationships and make predictions about which residues to mutate, the secondary structure for this protein family has been predicted by submitting the aligned sequences shown in FIG. 14 to the PredictProtein server (PredictProtein@EMBL-Heidelberg,DE). This method of prediction uses a neural net and preserves the information content of the aligned sequences, as well as that of surrounding residues rather than using only a single consensus residue at each position (Rost and Sander, 1993). These predictions (with an 82% level of confidence) are given in the last row of FIG. 14 and are consistent with analyses by the Raman and circular dichroic spectroscopies discussed below.

Interestingly, the putative active site cysteine at position 90 in UCH-L1 (see below) is flanked by two putative hydrophobic β-sheet regions. These two regions of β-sheet may span from the surface of the molecule to a more protected site deeper in the molecule and position the active site thiol in the expected catalytic cleft. The cysteine is juxtaposed between the very small residues, alaine and glycine. They may allow the approach of a scissile peptide bond to form the tetrahedral intermediate. If the inventors presume the mechanism of this protease to be papain-like. then there must also be a conserved histidine which can act as a catalytic base, polarizing the sulfhydryl and enhancing its nucleophilicity. Two positions in the UCH family have conserved histidines, these being positions 97 and 161 in UCH-L I. H97 is unlikely to be involved since it is only seven residues removed from the active site and at the opposite end of the predicted β-sheet. In contrast to papain and the serine proteases, thiol proteases of the interleukin-converting enzyme (ICE) family do not position a third residue to hydrogen bond to the catalytic histidine (Walker *et al.*, 1994). Thus, it is not known if a "catalytic triad" is involved in catalysis by the UCH gene family.

UCH Expression and Purification. Recombinant proteins were expressed in *E. coli* using a modified pRSET vector (Invitrogen). The modification removed the coding region for the oligohistidine leader sequence present in the parent vector. Expression in this system is driven by a T7 RNA polymerase promoter, with induction of the polymerase by IPFG. Upon induction, the UCH isozymes and

mutants were expressed at 15%-30% of the soluble protein. The enzymes were expressed, purified (see Experimental Procedures), and assayed for kinetic parameters.

To identify the active site residues involved in UCH catalysis, the inventors have mutagenized the wild type UCH-L1 cDNA. The vector encoding this UCH isozyme is more tractable for mutagenesis (compared to UCH-L3) because of its greater number of useful restriction sites. Several mutants were made whose properties are summarized in Table 5. In every case, UCH-L1 mutant proteins were produced in amounts equal to the wild type enzyme, based on SDS-PAGE analysis of expression lysates. The inventors assayed each expression lysate for activity, and active mutants were purified as described.

TABLE 5:

Mutagenesis and Kinetics of UCH-L1 Mutants[a]

Mutant	Codon Change	Relative Rate (Velocity/wt Velocity)	K_m (μM)
wild type		1.00	1.20
C90S	TGT → TCT	$<1 \times 10^{-7}$	nd
H97Q	CAC → CAA	0.85	0.65
H97N	CAC → AAC	0.87	0.60
HI61D	CAT → GAC	8.5×10^{-5}	1.50
H161K	CAT → AAA	$<1 \times 10^{-7}$	nd
H161N	CAT → AAC	$<1 \times 10^{-7}$	nd
H161Q	CAT → CAA	$<1 \times 10^{-7}$	nd
H161Y	CAT → TAC	$<1 \times 10^{-7}$	nd
D176N	GAT → AAT	0.025	7.40
Q73R	CAA → CGA	0.97	1.10

[a]Active mutants were purified as described for the wild type enzyme (see Experimental Procedures). Hydrolysis rates are the average of two determinations at 15 μM UbOEt, or were Michaelis constants determined according to Wilkinson *et al.* (1986). Wild type UCH-L1 velocity is

25 μmol/min/mg vs ubiquitin ethyl ester (nd: not determined).

Identification of the Active Site Cysteine. The inventors examined the effect of changing the putative active site thiol (C90) to a serine. This cysteine residue is conserved among all UCH's and was suspected to be involved in catalysis. though direct proof of this residues role in catalysis has not yet been shown. The inventors generated a UCH-L1-C90S mutant (see Procedures). Assay of the bacterial lysate expressing UCH-L1-C90S showed no detectable activity. To quantitatively assess the upper limit of this activity, the C905 mutant was purified and assayed. Even at equimolar enzyme to substrate ratio (17 μM), the half-life of the substrate is over 4.5 h. Because serine is isoelectronic with cysteine, it is likely that this abrogation of activity is a direct effect and not the result of a structural change. In support of this, the C905 mutant exhibits a thermal denaturation profile with thermodynamic parameters nearly identical to the native enzyme (see below). Therefore, cysteine 90 is directly involved in catalysis, probably as the active site nucleophile.

Identification of an Active Site Histidine. The inventors next sought to identify the active site histidine. Two positions in the alignment have a conserved histidine, corresponding to H97 and H161 in UCH-L1. To determine if these were important to catalytic function, the inventors conservatively mutated H97 to a glutamine or asparagine. These carboxamide residues cannot provide a general base for catalytic function, but could provide hydrogen bonding similar to the N1 or N3 imidazole nitrogens and hence could provide a structural replacement. Purified UCH-L1 H97Q and H97N catalytic velocities are approximately 85% as rapid as the wild type enzyme (Table 5 and Experimental Procedures). This suggests that H97 is not involved in catalysis.

The inventors then mutated the other fully conserved histidine at position 161. In short, all H161 mutants were either catalytically inactive or very significantly impaired. H161Q, H161N, H161Y, and H161K possess no measurable esterase

activity down to the detection limit of the inventors' assay. These mutants are minimally seven orders of magnitude slower than the wild type hydrolase. Individual H161 mutations could be expected to supply an adequate structural replacement for positive electrostatic charge (lysine), hydrogen bonding by the imidazoie π (asparagine) and τ (glutamine) nitrogens (Vaaler and Snell, 1989), or aromaticity and steric volume (tyrosine). Interestingly, UCH-L1 H161D shows detectable activity, about 4 orders of magnitude less than that of native enzyme. Determination of the K_m of this purified mutant showed that only the reaction rate was altered and that the K_m was unchanged (Table 5). In this context a carboxylate may function as a general base or a hydrogen bond acceptor. Either interaction would abstract proton density from the nearby cysteine thiol and enhance its nucleophilicity. Since neither H161N nor H161Q can support this level of catalysis, but could hydrogen bond, the inventors favor a direct role for D161 as a general base. This would be the first example of a functional cys—asp dyad in a protease, though the velocity of catalysis is small. UCH-L1 H161D shows CD spectra typical of native UCH-L1 (described below), suggesting that this residue is not important for the gross enzyme structure. The inventors' data therefore indicate that histidine 161 is intimately involved in catalysis, probably as an active site general base.

Mutation of the ELDGR Box. Because the binding of ubiquitin to UCH is primarily electrostatic (shown below), and since acid residues may be involved in catalysis, the inventors mutated a universally conserved aspartate in the most conserved area of the UCH sequence, the ELDGR box. D176 was changed to an asparagine, resulting in a sterically unaltered charge mutant in a highly conserved region. This mutant shows a significant, measurable activity of 2.5% wild type. To determine if the drop in catalytic rate was due to an effect on binding strength, the K_m was determined (Wilkinson *et al.*, 1986). Progress curve kinetics (Wilkinson *et al.*, 1986; Orsi and Tipton, 1979) show this mutant to have a K_m = 7.4 μM, approximately 6-fold weaker than that of the native enzyme. The inventors find that the calculated specificity constant V_m/K_m is 250-fold lower than the wild type L1. Catalytic efficiency is thus lowered dramatically, but is not obliterated, and this might be

expected for a residue not directly involved in catalysis. The ELDGR box may therefore be involved in the formation of a binding site or the orientation of the substrate.

Mutation of Q73. The amplification of the UCH-L1 coding sequence by RT-PCR™ resulted in two errors. One of these changes, a G to C transversion affecting V20O, was silent and was not repaired. Another G to A transition generated the mutant Q73R. The inventors repaired the R73 mutation by replacing the defective region with a fragment from the rat UCH-L1 cDNA. Both rat and human proteins have identical sequences in this region (Kajimoto *et al.*, 1992; Day *et al.*, 1990), and the swap thus repaired the original PCR™ mutation (Experimental Procedures). Residue 73 is 17 residues N-terminal to the active site cysteine. It is predicted to be at the surface of the enzyme, possibly as part of a turn at the opposite end of the β-sheet anchoring the active site cysteine. Since all known UCH sequences have a Q in this region (equivalent to either position 73 or 74 in UCH-L1), it was of interest to examine the catalytic activity of this mutant. Table 1 shows that mutation of this position to the positively charged R residue had no effect on the activity of the enzyme or its affinity for substrate.

Structural Effects of These Mutations. To ensure that the lack of activity in these mutants was not due to gross structural misfolding of the enzymes, the inventors analyzed selected mutants by circular dichroism. UCH-L1 mutants Q73R, H161D, D176N, and C90S all show CD spectra typical of UCH-L1 (described below), suggesting that these residues are not important for the gross enzyme structure. All of these mutants were expressed at levels similar to the wild type, again suggesting that folding and solubility were not problems with these specific mutations.

Binding of Ubiquitin to UCH-L3. To characterize the ubiquitin binding site and to identify any structural changes and/or perturbation of the environment of amino acid side chains associated with the binding of ubiquitin to UCH, the inventors have studied the spectral properties of the more soluble isozyme, UCH-L3, upon binding of

ubiquitin. Circular dichroism has previously been used to monitor protein-protein interactions accompanied by conformational changes, as well as to examine the environment of aromatic residues (Beltramini *et al.*, 1992; Blazy *et al.*, 1992; Grobler *et al.*, 1994; Vuillemier *et al.*, 1993). The inventors purified UCH-L3 (Experimental Procedures) and used it to study substrate binding by various approaches. The inventors were unable to detect any changes of ellipticity in CD difference spectra upon binding of ubiquitin and UCH-L3. This suggests that the structure of the two proteins are not altered by binding, such that no gross "induced fit" conformational changes are detectable.

To examine if aromatic residues were perturbed by substrate binding, the UV spectra of Ub and UCH-L3 were recorded in dual-compartment cells. After the compartment contents were mixed to initiate binding, no significant spectral change was seen relative to the unmixed control. The data from UV and CD spectra cannot distinguish minor tertiary structure alterations in UCH, and the inventors cannot comment on this possibility solely on their basis. The data do suggest that the electronic environments of the aromatic side chains are not radically altered by ubiquitin binding. Above 340 nm, the lack of UV absorption is consistent with the absence of chromophoric prosthetic groups in the enzyme. The spectra of UCH-L3 yield a Beer-Lambert extinction coefficient of 21 000 L/tool cm at 280 nm. UCH-L1 exhibits similar spectral characteristics, with an extinction of 15 600 L/tool cm. These data are consistent with the expected extinction based on the aromatic content of the polypeptides.

Binding of ubiquitin to UCH-L3 was not detectable by any of the spectral methods used above. Nonetheless, kinetic evidence predicts a sub-micromolar binding constant (Wilkinson *et al.*, 1986). Additionally, it is known that the enzyme is specifically bound to and eluted from a ubiquitin affinity column (Duerksen-Hughes *et al.*, 1989; Pickart and Rose, 1985). The kinetically obtained K_m must not be interpreted as a substrate dissociation constant, and the ubiquitin affinity column cannot be used to quantify the binding strength. Thus a direct gel filtration

approach was used to monitor this binding. In these studies, the column buffer is equilibrated with ligand ubiquitin and the enzyme sample is supplemented with an equal concentration of ligand. If binding occurs, one expects to observe a peak of ligand at the elution position of the enzyme and a depressed level of ligand at the included volume of the column. FIG. 15A shows that purified UCH-L3 is 91% occupied by ubiquitin when chromatographed in the presence of 5 μM ubiquitin-containing buffer. Integration of the peak area shows that 3.45 nmol of ubiquitin was bound to the 3.80 nmol of UCH-L3 applied. An apparent binding constant of 0.5 μM can be calculated from these data. This is similar to the K_m for UbOEt (Wilkinson *et al.*, 1986) and implies that most of the binding energy is due to ubiquitin alone and not the ester functionality. These data demonstrate that UCH-L3 possesses only one binding site with a micromolar K_d and that the stoichiometry of binding is 1:1.

The above data demonstrate the binding of ubiquitin to UGH-L3 and suggest that there are few gross structural changes associated with this binding. Further, the environment of aromatic residues is not greatly perturbed. This suggests that polar interactions may be important for the binding. Indeed, the inventors have noted that increased ionic strength inhibits hydrolysis of ubiquitin ethyl ester. This inhibition is virtually complete at 10 μM substrate and 0.40 M NaCl. To examine if the inhibition by ionic strength was due to decreased substrate binding, or to a change in the catalytic properties of the protein, the inventors have repeated these binding studies in the presence of inhibitory levels of salt. Ubiquitin binding is completely abrogated in the presence of 0.5 M NaCl (FIG. 15B). The structure of the enzyme is not grossly perturbed by the presence of salt, as the CD spectra of UCH-L3 in 0 and 0.5 M NaCl are virtually identical. These data suggest that the binding interactions of the enzyme and substrate are primarily electrostatic and not hydrophobic.

Spectroscopic: Analysis of UCH-L3. Circular dichroism spectroscopy was used to estimate the amount of secondary structure motifs in UCH isozymes and mutants (FIG. 16) and to evaluate the effects of mutation on the folded protein

structure. The CD spectra show an absolute minima at 222 nm, characteristic of the presence of α-helices. This is also confirmed by the relative minima at 208 nm and absolute maxima at 202 nm (Johnson, 1988). Calculating the mean residue ellipticity, at 208 and 222 nm, the inventors obtain values of 12 090 and 9160 deg cm^2/dmol, respectively. Using the sum of structures constraint (Greenfield and Fasman, 1969) these values predict α-helix contents of 31.2% and 32.4%. Also shown in FIG. 16 is the near-UV dichroism due to the chiral environment of the aromatic residues (curve labeled × 100). As is typical, this region shows much less ellipticity (~60 deg cm^2/dmol), but since this region might serve as an environmentally sensitive reporter for the aromatic residues the inventors have shown it.

Finally, classical nonresonance laser Raman spectroscopy was also used as a structural probe. FIG. 16 shows the Raman spectra of UCH-L3 from 400 to 1750 cm^{-1}. The inventors used two methods to calculate the amounts of structural motifs which are based on the conformationally sensitive nature of the peptide carbonyl stretch absorbance. The spectral bandwidth, intensity, and position of this amide I Stokes emission were used to estimate quantities of four generic secondary structures: helix, β-sheet, turn, and random (Alix *et al.*, 1981). This method suggests 48% helical content, 25% β-sheet, 16% turn, and 11% "other". Another method (Lippert *et al.*, 1976) uses the spectral characteristics (1240, 1632, and 1660 cm^{-1} transitions) of pure helix, β-sheet, and random forms of poly L-lysine to calculate the secondary structure content. The inventors' data predict 40% helix, 43% β-sheet, and 17% random coil when analyzed in this way, but this method cannot distinguish between β-turn and β-sheet motifs. These predictions therefore concur generally with predictions based on Alix *et al.* (1988) and also with the CD data presented above. Weighting the Raman, CD, and prediction algorithms equally, approximate averages of 38% helix, 22% β-sheet, 18% turn or loop, and 19% "nonordered" secondary structures are obtained. Minor discrepancies between the methods may arise as a consequence of the "sum of structure" constraints or from the nature of the model compounds used as the basis for the various computations described above.

The inventors' data show that UCH isozymes possess both helix and β-sheet motifs, similar to the papain family of thiol proteases. To date, the solution crystal structures of five thiol proteases have been solved. Three of these, papain, calotropin D1, and actinidin, are from plant sources; two others, liver cathepsin B and the interleukin 1-β-converting enzyme "ICE", are from mammalian sources [reviewed by Walker *et al.* (1994). These enzymes differ from the all-β-chymotrypsin class of serine proteases in both catalytic residues and overall structure. ICE and subtilisin both possess helical content, however, and exhibit an antiparallel β-sheet core domain. While UCH enzymes resemble the papain family members in size and secondary structure content, sequence comparison with the papain family suggests that the UCH family should be classified as a distinct gene family. The solution of a UCH crystal structure would provide a valuable addition to the small collection of α/β-proteases, and these studies are ongoing.

Thermal Denaturation. The above results demonstrate that the recombinant enzymes and mutants display normal spectroscopic properties at room temperature. This suggests that all mutants tested fold correctly and are soluble under these conditions. However, the temperature of the enzymatic assay and normal physiological environment of these enzymes is 37°C. To demonstrate that the loss of activity was due to a direct effect and not irreversible unfolding of the enzymes at assay temperature, the inventors have conducted thermal denaturation studies monitoring the 222 nm circular dichroism signal. Using this technique, the thermodynamics of protein denaturation have been studied for several enzymes Alexander *et al.,* 1992; reviewed by Privalov and Gill (1988). This method provides a powerful, general tool for assessing the structural stability of enzymes and mutants. FIG. 17 shows the temperature-dependent changes in the 222 nm CD signal of UCH-L1. As can be seen, there is a thermal transition at approximately 52°C resulting in a 45% diminishment in this conformationally sensitive signal. UCH-L3 is also subject to the same transition, though the loss of ellipticity is slightly less. Cooling the sample results in the restoration of the original spectra, and wavelength scans at 65°C are typical of proteins with high random coil content. Also, the

transition is fully reversible if the protein concentration is less than 100 μg/mL (10 μg/mL for H161K and H161Y) and if the protein is not allowed to remain denatured for more than 5 min before the temperature is lowered.

These data can be analyzed according to a two-state model, and the relevant thermodynamic parameters can be calculated. The inset to FIG. 17 shows the Arrhenius plot of the data. As obtained from the replot, this transition is characterized by values of ΔH = 1.56 kJ/mol of residue, ΔS = 4.80 J/K mol of residue, and ΔG = 28.6 kJ/mol of UCH-L1 at 25°C. It is assumed that this transition is the reversible denaturation of UCH. The rather modest stability, of this protein is consistent with the reversible folding of a single domain protein. Many small globular proteins exhibit folded states stabilized by only 20-60 kJ/mol of Gibbs free energy (Privalov, 1979).

TABLE 6

Thermodynamics of Denaturation of UCH's[a]

Enzyme	Melting Point (T_m, ±0.2°C)	Enthalpy, ΔH (kJ/mol of aa)	Entropy, ΔS (J/K mol of aa)	Gibbs Energy, ΔG (kJ/mol of UCH)
UCH-L3	50.9	1.15	3.52	21.7
UCH-L1	51.8	1.56	4.80	28.6
UCH-L1 C90S	51.5	1.55	4.78	27.7
UCH-L1 H161D	49.9	1.50	4.69	22.6
UCH-L1 H161K	52.7	1.07	3.30	19.1
UCH-L1 H161Y	52.7	1.10	3.40	19.2

[a]Melting points are derived from the primary denaturation data. Thermodynamic values for denaturation are calculated as described in the text, where ΔH = kJ/mol of amino acid residue, ΔS = J/K mol of amino acid residue, and ΔG = kJ/mol of UCH at 25°. Conventions are according to Privalov (1979).

The inventors also performed this thermodynamic analysis for the UGH-L3 isozyme and the L1 isozyme mutants C90S, H161D, H161K, and H161Y. In general

the wild-type and mutant enzymes have virtually indistinguishable circular dichroism spectra and only slightly differing denaturation curves. All denature at 50-53°C, where the melting point is defined as that point in the thermal denaturation curve where $K_{eq} = 1$, *i.e.*, the midpoint. Thermodynamic values thus derived are shown in Table 6. By comparison, the neuron specific UCH-L1 appears slightly more stable than its hemopoeitic homologue, UCH-L3. Wild type L1 and the isoelectronic mutant C90S both show virtually identical melting points and thermodynamic stabilities per residue (Privalov and Gill, 1988) with ΔG: 28.6 and 27.7 kJ/mol at 25°C, respectively. Mutations at the catalytic histidine were only slightly destabilizing. as determined by a melting point depression (H161D) or unfavorably altered thermodynamic state functions (H161K and H161Y). On the basis of these data, the inventors conclude that the inactivity of the C90 and H161 mutants is due to the loss of important catalytic residues and not due to misfolding or a decreased stability of the folded form.

Given the above observations, the present invention also contemplates constructing columns containing a matrix material that has immobilized enzyme bound to its surface. Such a column may be constructed such that it is much more likely than not that the immobilized enzyme, whether UCH-L1, UCH-L3, or variants thereof, shows greater catalytic activity as opposed to unbound enzyme. Also contemplated is protecting the face of the enzyme utilizing a cross-linked ubiquitin-protein or ubiquitin-peptide complex. The binding of such a complex to the enzyme is generally tight and specific, and if cross linked, the complex serves as a protector of the active site. To activate the enzyme, one may disassociate the ubiquitin with, for example, a high salt concentration. Such columns will find use in catalytically cleaving and then separating ubiquitin from the peptides and small proteins that are part of the fusion protein. Conversely, if one wanted to further purify the enzyme, one could employ a column having ubiquitin bound to its matrix, and more preferably, ubiquitin that has been cross linked.

The inventors have presented data to demonstrate that cysteine 90 and histidine 161 are the active site nucleophile and general base involved in UCH-L1 catalysis. These data assist in crystalographic model building, as the two residues must be juxtaposed in the tertiary structure and will define the active site. It can also be safely assumed that the other isozymes of the UCH family possess the same catalytic chemistry and residues, for reasons described above. The electronic nature of the binding suggests that one of two faces of ubiquitin is involved in an extensive interaction with this enzyme. One face has been defined as an "acidic face" with many such clustered on the surface of the α-helix from residues 20 to 34. Many of the basic residues are clustered on the opposite face of the molecule. It is not immediately obvious which face is contacting the surface of the enzyme, although there are several approaches which could be pursued to further define this. It is interesting to note that the majority of amino acid substitutions across species occur in the "acidic" face of ubiquitin (Wostmann *et al.*, 1992), and for this reason, the inventors assume that the "basic" face is involved in these binding interactions. Data from Burch and Haas (1994) suggest that R42 of ubiquitin is involved in recognition

by UCH-L3. Also, the aspartate in the conserved ELDGR box may be involved in the binding. The effect of the D176N mutation on the Michaelis constant for ubiquitin shows that this residue may participate in an ionic interaction with ubiquitin or provide minor "orienting" effects for the fine tuning of substrate positioning. Rose and Warms (1983) have also shown that the two C-terminal glycine residues are necessary for effective inhibition of UCH-L3 by ubiquitin. The inventors find that the attachment of a hexahistidine motif to the N-terminus of ubiquitin does not affect hydrolysis rates to any measurable extent.

In summary, the inventors' data suggest that UCH isozymes (a) utilize cysteine 90 as the nucleophile, (b) use histidine 161 as the general base catalyst, (c) bind ubiquitin electrostatically, (d) bind the intact ubiquitin C-terminus, (e) may possess a carboxylate P3 binding pocket for arginine, (f) do not bind the amino terminus of ubiquitin, (g) bind other basic residues in ubiquitin, and (h) utilize several of UCH's acidic residues in binding. These studies are useful in building models of the enzyme for crystallographic and structural studies, for defining the enzyme-substrate interaction, and in site-directed mutagenesis studies designed to alter recognition and specificity of these enzymes.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Bacon and Anderson, "A fast algorithm for rendering space-filling molecule pictures," *J. Molec. Graphics*, 6:219-220, 1988.

Baker, Tobias, Varshavsky, "Ubiquitin-specific proteases of Saccharomyces cerevisiae: cloning of UBP2 and UBP3, and functional analysis of the UBP gene family," *J. Biol. Chem.*, 267:23364-23375, 1992.

Beers and Callis, *J. Biol. Chem.*, 268:21645-21649, 1993.

Bradbury, "Reversible histone modifications and the chromosome cell cycle," *Bioessays*, 14:9-16, 1992.

Brünger, "Free R value: a novel statistical quantity for assessing the accuracy of crystal structures," *Nature*, 355:472-475, 1992a.

Brünger, "X-PLOR version 3.1, a system for X-ray crystallography and NMR," New Haven, CT: Yale University Press, 1992b.

Carmona, Dufour, Plouffe, Takebe, Mason, Mort, Menard, "Potency and selectivity of the cathepsin L propeptide as an inhibitor of cysteine proteases," *Biochem.*, 35:8149-8157, 1996.

CCP4, The CCP4 suite: programs for protein crystallography," *Acta Cryst.*, 50(D):760-763, 1994.

Chau, Tobias, Bachmair, Marriott, Ecker, Gonda, Varshavsky, "A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein," *Science*, 243:1576-1583, 1989.

Chen and Pickart, *J. Biol. Chem.*, 265:21835-21842, 1990.

Chen, Parent, Maniatis, "Site-specific phosphorylation of IκBα by a novel ubiquitination-dependent protein kinase activity," *Cell*, 84:853-862, 1996.

Ciechanover and Schwartz, "The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins," *FASEB J.*, 8:182-191, 1994.

Coulombe, Grochulski, Sivaraman, Ménard, Mort, Cygler, "Structure of human procathepsin L reveals the molecular basis of inhibition by the prosegment," *EMBO J.*, 15:5492-5503, 1996.

Cowtan, 'dm': "An automated procedure for phase improvement by density modification," *Joint CCP4 and ESF-EACBM newsletter on protein crystallography*, 31:34-38, 1994.

Cygler, Sivaraman, Grochulski, Coulombe, Storer, Mort, "Structure of rat procathepsin B. Model for inhibition of cysteine protease activity by the proregion," *Structrue*, 4:405-416, 1996.

Drenth, Kalk, Swen, "Binding of chloromethyl ketone substrate analogues to crystalline papain," *Biochem.*, 15:3731-3738, 1976.

Finley, Bartel, Varshavsky, *Nature,* 338:394-401, 1989.

Glichrist, *In: Heterocyclic Chemistry*, 2nd Ed., John Wiley & Sons, New York, Ch. 8, pp 314-316, 1992.

Gregori, Poosch, Cousins, Chau, "A uniform isopeptide-linked multikbiquitin chain is sufficient to target substarte for degradation in ubiquitin-mediated protolysis," *J. Biol. Chem.*, 265:8354-8357, 1990.

Haas, Katzung, Reback, Guarino, "Functional characterization of the ubiquitin variant encoded by the baculovirus Autographa californica," *Biochem.*, 35:5385-5394, 1996.

Henchoz, De Rubertis, Pauli, Spierer, "The dose of a putative ubiquitin-specific protease affects position-effect variegation in Drosophila melanogaster," *Mol. Cell. Biol.*, 16:5717-5725, 1996.

Hershko and Ciechanover, "The Ubiquitin System for Protein Degradation," *Annu. Rev. Biochem.*, 61:761-807, 1992.

Hicke and Riezman, "Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis," *Cell*, 84:277-287, 1996.

Holm and Sander, "Protein structure comparison by alignment of distance matrices," *J. Mol. Biol.*, 233:123-138, 1993.

Huang, Baker, Fischer-Vize, "Control of cell fate by a deubiquitinating enzyme encoded by the *fat facets* gene," *Science*, 270:1828-1831, 1995.

Hutchinson and Thornton, "PROMOTIF - A program to identify and analyze structural motifs in proteins," *Prot. Sci.*, 5:212-220, 1996.

Jentsch, "Ubiquitin-dependent protein degradation: a cellular perspective," *Trend. Cell Biol.*, 2:98-103, 1992.

Johnston, Larsen, Cook, Wilkinson, Hill, *EMBO J.*, 16:3787-3796, 1997.

Jones, Zou, Cowan, Kjeldgaard, "Improved methods for building protein models in electron density maps and location of errors in these models," *Acta Cryst.*, 47(A):110-119, 1991.

Jonnalagadda, Butt, Marsh, Sternberg, Mirabelli, Ecker, Crooke, *J. Biol. Chem.*, 262:17750-17756, 1987.

Kamphuis, Kalk, Swarte, Drenth, "Structure of papain refined at 1.65 Å resolution," *J. Mol. Biol.*, 179:233-256, 1984.

Karrer, Peiffer, DiTomas, "Two distinct gene subfamilies within the family of cysteine protease genes," *Proc. Natl. Acad. Sci. USA*, 90:3063-3067, 1993.

Kleywegt and Jones, "A super position," *Joint CCP4 and ESF-EACBM newsletter on protein crystallography*, 31:9-14, 1994.

Kraulis, "Molscript: a program to produce both detailed and schematic plots of protein structures," *J. Appl. Cryst.*, 24:946-950, 1991.

Larsen, Price, Wilkinson, "Substrate binding and catalysis by ubiquitin C-terminal hydrolases: Identification of two active site residues," *Biochem.*, 35:6735-6744, 1996.

Laskowski, MacArthur, Moss, Thornton, "PROCHECK: a program to check the stereochemical quality of protein structures," *J. Appl. Cryst.*, 26:283-291, 1993.

Liu, Miller, Kohr Jr., Silber, "Purification of a ubiquitin protein peptidase from yeast with effecient *in vitro* assays," *J. Biol. Chem.*, 264:20331-20338, 1989.

Matthews, "Solvent content of protein crystals," *J. Mol. Biol.*, 33:491-497, 1968.

Matunis, Coutavas, Blobel, "A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex," *J. Cell. Biol.*, 135:1457-1470, 1995.

McRee, "A visual protein crystallographic software system for X11/XView," *J. Molec. Graph.*, 10:44-46, 1992.

Ménard, Carrière, Laflamme, Plouffe, Khouri, Vernet, Tessier, Thomas, Storer, "Contribution of the glutamine 19 side chain to transition-state stabilization in the oxyanion hole of papain," *Biochem.*, 30:8924-8928, 1991.

Meyer, Cole, Radhakrishnan, Epp, "Structure of native porcine pancreatic elastase at 1.65 Å resolution," *Acta Crystallogr.*, 44(B):26-55, 1988.

Miller, Henzel, Ridgeway, Kuang, Chisholm, Liu, "Cloning and expression of a yeast ubiquitin-protein cleaving activity in *E. coli*," *Biotech.*, 7:698-704, 1989.

Moazed and Johnson, "A deubiquinating enzyme interacts with SIR4 and regulates silencing in S. cerevisiae," *Cell*, 86:667-677, 1996.

Monia, Ecker, Jonnalagadda, Marsh, Gotlib, Butt, Crooke, *J. Biol. Chem.*, 264:4093-4103, 1989.

Narasimhan, Potter, Haas, "Conjugation of the 15-kDa interferon-induced ubiquitin homolog is distinct from that of ubiquitin," *J. Biol. Chem.*, 271:324-330, 1996.

Nicholls, Sharp, Honig, "Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons," *PROTEINS, Structure, Function and Genetics*, 11:281-296, 1991.

O'Hara, Hemmings, Buttle, Pearl, "Crystal structure of glycyl endopeptidase from carica papaya: A cysteine endopeptidase of unusual substrate specificity," *Biochem.*, 34:13190-13195, 1995.

Olvera and Wool, "The carboxyl extension of a ubiquitin-like protein in rat ribosomal protein S30," *J. Biol. Chem.*, 268:17967-17974, 1993.

Otwinowski, "Maximum likelihood refinement of heavy atom parameters," *In: Isomorphous replacement and anomalous scattering*, (ed.) Evans, Leslie, (Daresbury Lab.: Warrington WA4 4AD, England), pp. 80-86, 1991.

Otwinowski, "Oscillation data reduction program," *In: Data collection and processing*, (ed.) Sawyer, Isaacs, Bailey, (SERC Daresbury Laboratory: Warrington WA4 4AD, England), pp. 56-62, 1993.

Özkaynak, Finley, Solomon, Varshavsky, "The yeast ubiquitin genes: a family of natural gene fusions," *EMBO J.*, 6:1429-1439, 1987.

Papa and Hochstrasser, "The yeast DOA4 gene encodes a deubiquinating enzyme related to product of the human *tre*-2 oncogene," *Nature*, 366:313-319, 1993.

Pickart and Rose, "Ubiquitin carboxyl-terminal hydrolase acts on ubiquitin carboxyl-teminal amides," *J. Biol. Chem.*, 260:7903-7910, 1985.

Pickart and Rose, "Mechanism of ubiquitin carboxyl-terminal hydrolase: borohydride and hydroxylamine inactivate in the presence of ubiquitin," *J. Biol. Chem.*, 261:10210-10217, 1986.

Ramakrishnan and Biou, "Treatment of MAD as a special case of MIR," *Method. Enzymol.*, 1997 **(IN PRESS)**.

Rashin, Iofin, Honig, "Internal cavities and buried waters in globular proteins," *Biochem.*, 25:3619-3625, 1986.

Rawlings and Barrett, "Families of cysteine peptidases," *In: Method Enzymol.*, Barrett (Ed.), Academic Press, San Diego, 244:461-486, 1994.

Roff, Thompson, Rodriguez, Jacque, Baleux, Arenzana-Seisdedos, Hay, "Role of IκBα ubiquitination in signal-induced activation of NG-κB *in vivo*," *J. Biol. Chem.*, 271:7844-7850, 1996.

Rose and Warms, *Biochemistry*, 22:4234-4237, 1983.

Rost and Sander, "Improved prediction of protein secondary structure by use of sequence profiles and neural networks," *Proc. Natl. Acad. Sci. U.S.A.*, 90:7558-7562, 1993.

Sambrook, Fritsch, Maniatis, *In: Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Press, Cold Springs Harbor, **pp ?**, 1989.

Sanger, Nicklen, Coulson, *Proc. Natl. Acad. Sci. USA*, 74:5463-5467, 1977.

Schechter and Berger, "On the size of the active site of proteases," *Biochem. Biophys. Res. Commun.*, 27:157-162, 1967.

Schröder, Phillips, Garmen, Harlos, Crawford, "X-ray crystallographic structure of a papain-leupetin complex," *FEBS Lett.*, 315:38-42, 1993.

Storer and Ménard, "Catalytic mechanism in papain family of cysteine peptidases. *In: Method. Enzymol.*, Barrett (Ed.), Academic Press, San Diego, 244:486-500, 1994.

Studier and Moffatt, "Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes," *J. Molec. Biol.*, 189:113-130, 1986.

Tobias and Varshovsky, "Cloning and functional analysis of the ubiquitin-specific protease gene Ubp1 of Saccharomyces cerevisiae," *J. Biol. Chem.*, 266:12021-12028, 1991.

Turk, Podobnik, Kuhelj, Dolinar, Turk, "Crystal structures of human procathepsin B at 3.2 and 3.3 Å resolution reveal an interaction motif between a papain-like cysteine protease and its propeptide," *FEBS Lett.*, 384:211-214, 1996.

Turk, Podobnik, Popovic, Katunuma, Bod., Huber, Turk, "Crystal structure of cathepsin B inhibited with CA030 at 2.0 Å resolution: A basis for the design of specific epoxysuccinyl inhibitors," *Biochem.*, 34:4791-4797, 1995.

Vijay-Kumar, Bugg, Cook, "Structure of ubiquitin refined at 1.8Å resolution," *J. Molec. Biol.*, 194:531-544, 1987.

Wand and Wilkinson, **INCOMPLETE CITE**

Weber, Gittis, Mullen, Abeygunawardana, Lattmann, Mildvan, "NMR docking of a substrate into the X-ray structure of staphylococcal nuclease," *Proteins: Structure, Function, and Genetics*, 13:275-287, 1992.

Wilkinson, "Purification and structural properties of ubiquitin. *In: Ubiquitin*, Rechsteiner (Ed.), Plenum Press, New York, pp 5-38, 1988.

Wilkinson, "Ubiquitin carboxyl-terminal hydrolase. *In: A Handbook of Proteolytic Enzymes*, Barrett and Rawlings (Ed.), Academic Press, San Diego, **pp ?**, 1997, **(IN PRESS)**.

Wilkinson, Cox, Mayer, Frey, "Synthesis and characterization of ubiquitin ethyl ester, a new substrate for ubiquitin carboxyl-terminal hydrolase," *Biochem.*, 25:6644-6649, 1986.

Wilkinson, Deshpande, Larsen, "Comparisons of neuronal (PGP 9.5) and non-neuronal ubiquitin C-terminal hydrolases," *Biochem. Sec. Trans.*, 20:631-637, 1992.

Wilkinson, Lee, Deshpande, Duerksen-Hughes, Boss, Pohl, "The neuron-specific protein PGP9.5 is a ubiquitin carboxyl-terminal hydrolase," *Science*, 246:670-673, 1989.

Wilkinson, Tashayev, O'Connor, Larsen, Kasperek, Pickart, "Metabolism of the polyubiquitin degradation signal: Structure, mechanism and role and isopeptidase T," *Biochem.*, 34:14535-14546, 1995.

Zhu, Carroll, Papa, Hochstrasser, D'Andrea, "DUB-1, a deubiquinating enzyme with growth-suppressing activity," *Proc. Natl. Acad. Sci. USA*, 93:3274-3279, 1996.

WHAT IS CLAIMED IS:

1. A method of preparing a molecule that binds to a UCH-L3 protein but does not substantially bind to a variant UCH-L3 protein, comprising determining a three-dimensional structure of a UCH-L3 or variant UCH-L3 protein and designing a molecule that binds to a UCH-L3 protein, but that does not bind substantially to a variant UCH-L3 protein.

2. The method of claim 1, further comprising testing the designed molecule for binding to said UCH-L3 protein.

3. A method for the identification of a candidate inhibitor substance that inhibits UCH-L3 activity comprising the steps of:

a) contacting a cell expressing a UCH-L3 protein with a candidate inhibitor substance; and

b) comparing the properties of said cell with the growth of said cell in the absence of said candidate inhibitor substance;

wherein a change in the properties is indicative of said substance being an inhibitor of UCH-L3 activity.

4. The method of claim 3, wherein said UCH-L3 protein expressed is a variant UCH-L3 protein

5. The method of claim 3, wherein said candidate substance is a small molecule inhibitor.

6. The method of claim 5, wherein the small molecule inhibitor is a substituted isoxazole, heterocyclic aromatic compound; or a sugar-linked aromatic compound.

7. A method for the identification of a candidate inhibitor substance that inhibits UCH-L3 expression comprising the steps of:

a) contacting a cell expressing a UCH-L3 protein with a candidate inhibitor substance; and

b) comparing the expression of UCH-L3 of said cell with the expression of UCH-L3 of said cell in the absence of said candidate inhibitor substance;

wherein a decrease in the expression of UCH-L3 is indicative of said substance being an inhibitor of UCH-L3 expression.

8. The method of claim 7, wherein said candidate substance is a small molecule inhibitor.

9. The method of claim 8, wherein the small molecule inhibitor is a substituted isoxazole, heterocyclic aromatic compound; or a sugar-linked aromatic compound.

10. A method of preparing a molecule that binds to a UCH-L3 protein, comprising determining a three-dimensional structure of a UCH-L3 protein and designing a molecule that binds to a UCH-L3 protein.

11. The method of claim 10, wherein the molecule increases the stability of the UCH-L3 protein.

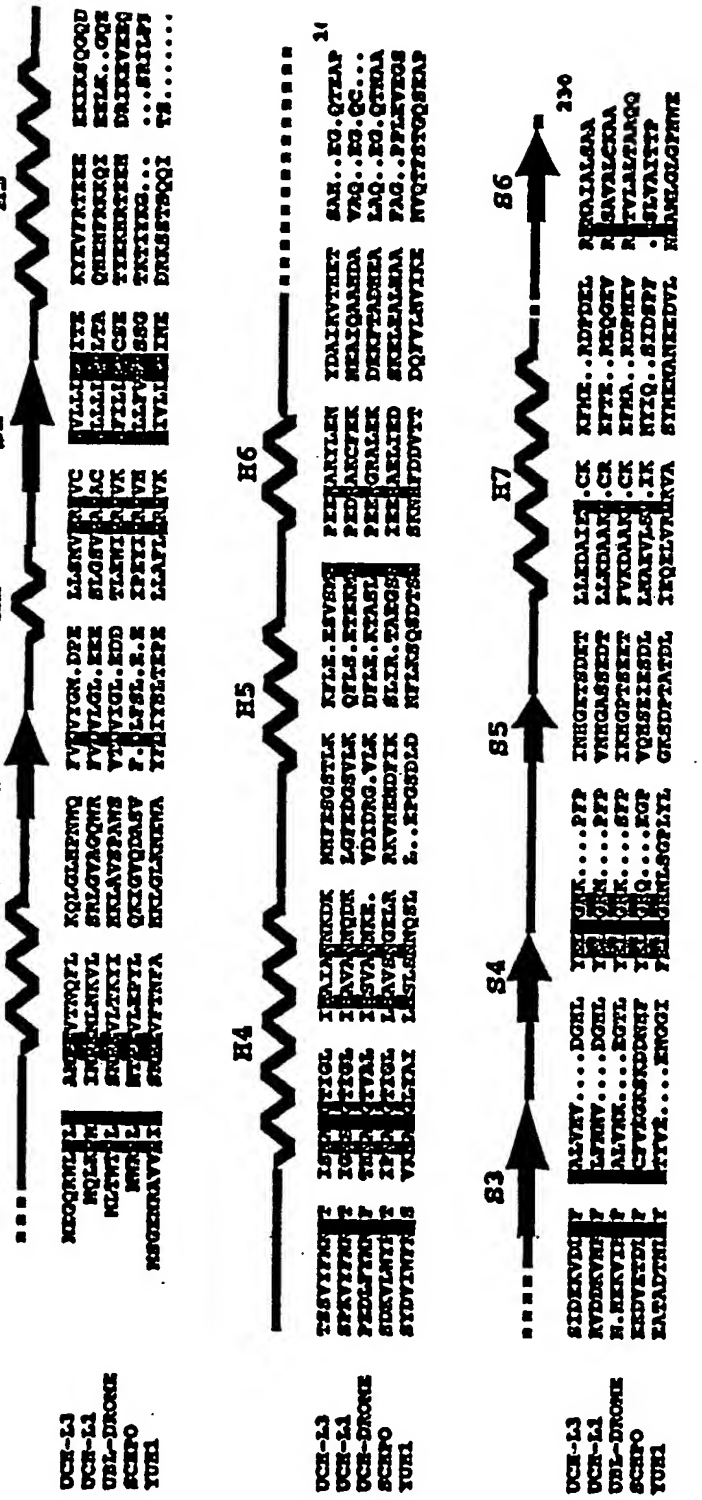
12. The method of claim 10, wherein the molecule decreases the stability of the UCH-L3 protein.

13. A variant UCH-L3 molecule having the properties of increased stability.

14. A process of regulating protein deubiquitination comprising exposing said protein to a deubiquitinating enzyme or a mutant deubiquitinating enzyme, which mutant does not catalyze the deubiquitination of said protein.

15. A variant UCH-L3 molecule having the properties of papain-like activity and deubiquitinating activity.

FIG. 1


H1
S1
H2
S2
H3
H4
H5
H6
S3
S4
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H7
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SCHPO
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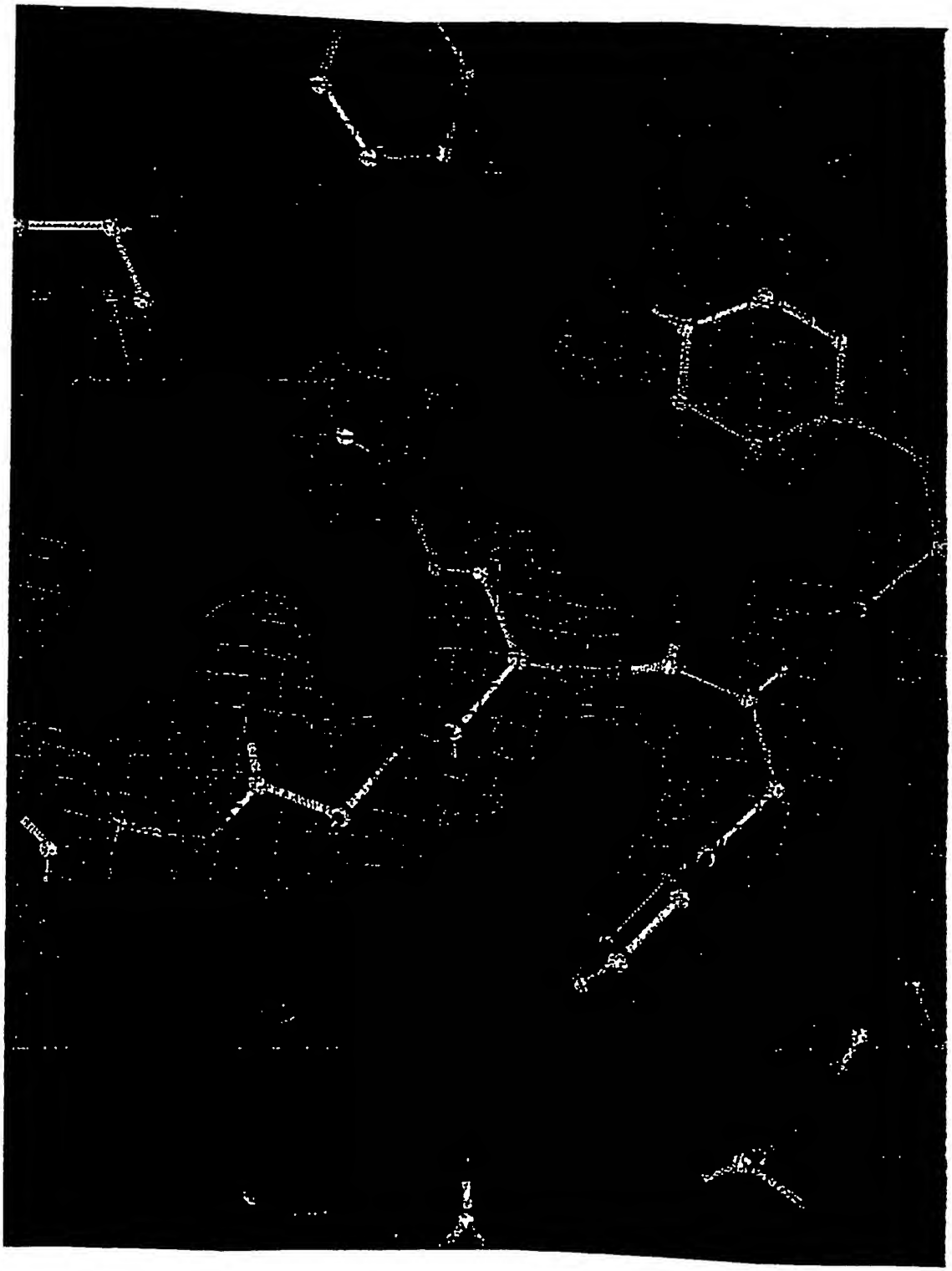
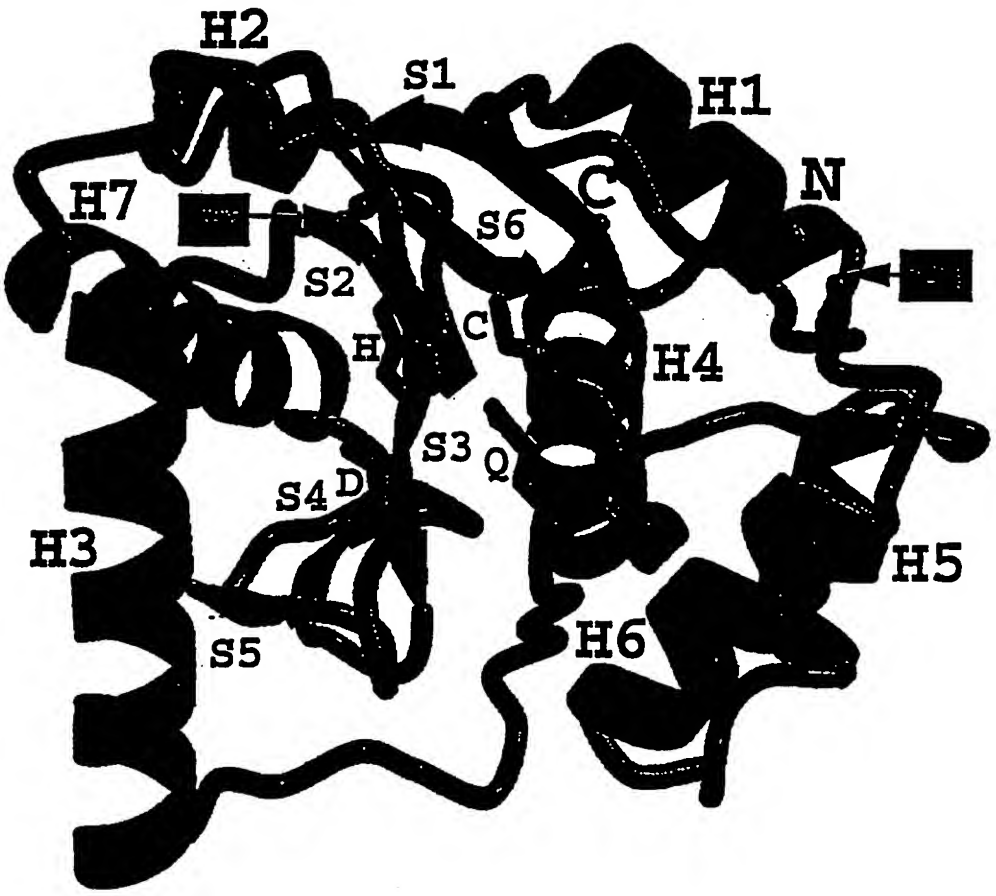



H2
S1
H1
H7
C
N
S6
S2
C
H
H4
S3
Q
S4
D
H3
H5
S5
H6

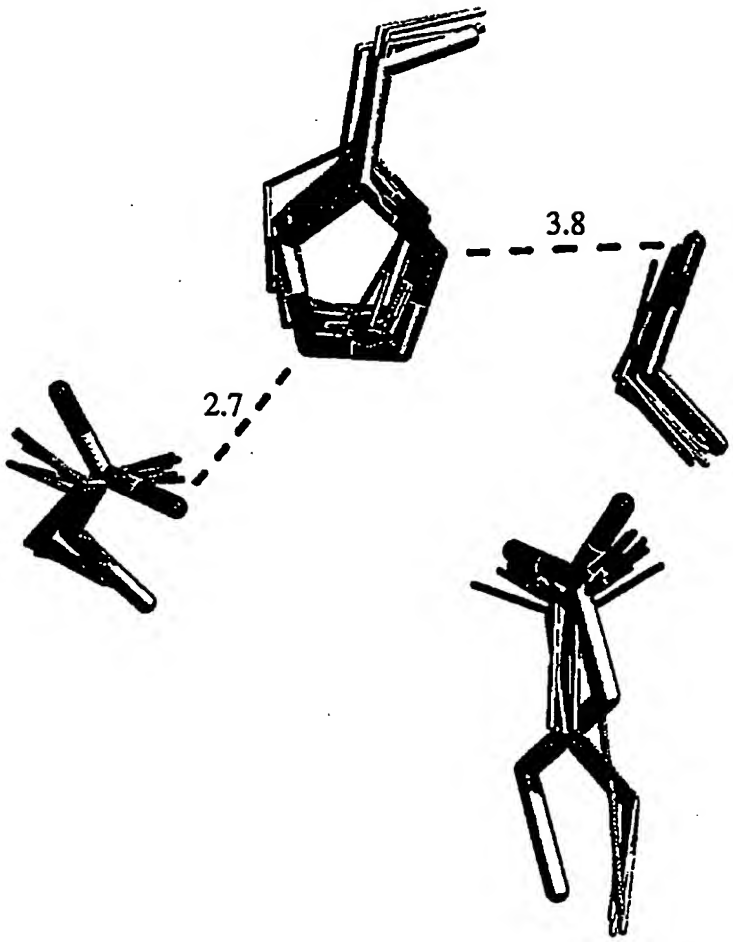

3.8
2.7

Fig. 5A

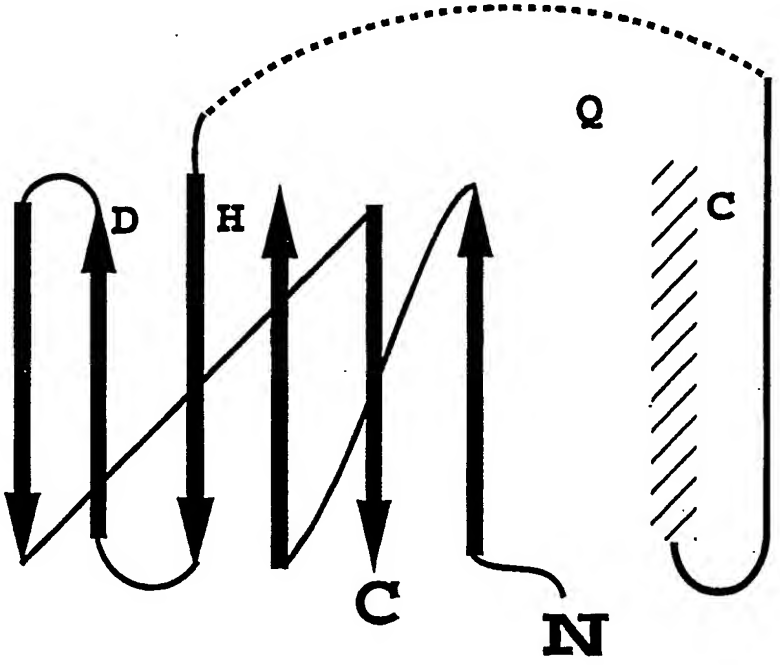
A

UCH-L3

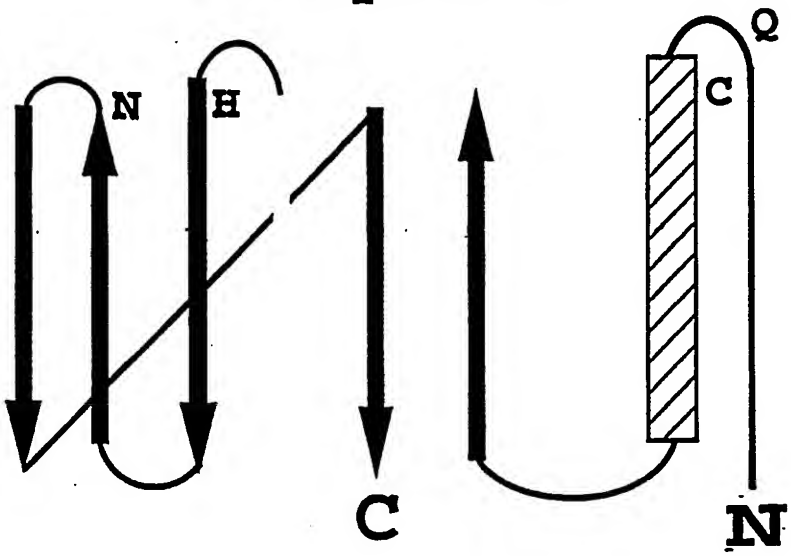
Cathepsin B

Fig. 5B

B **UCH-L3**



Cathepsin B



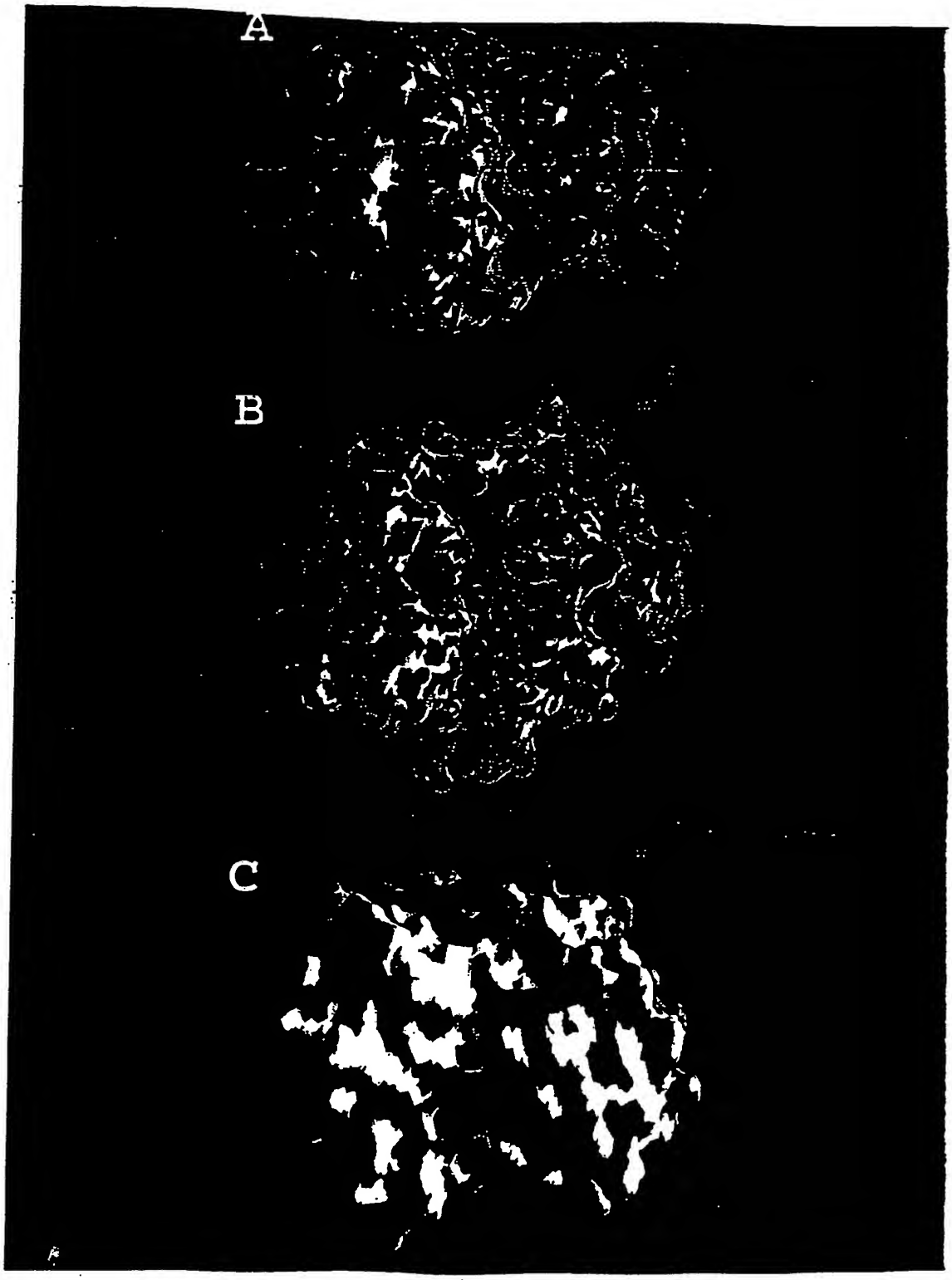

A
B
C

Fig. 1

Fig. 9

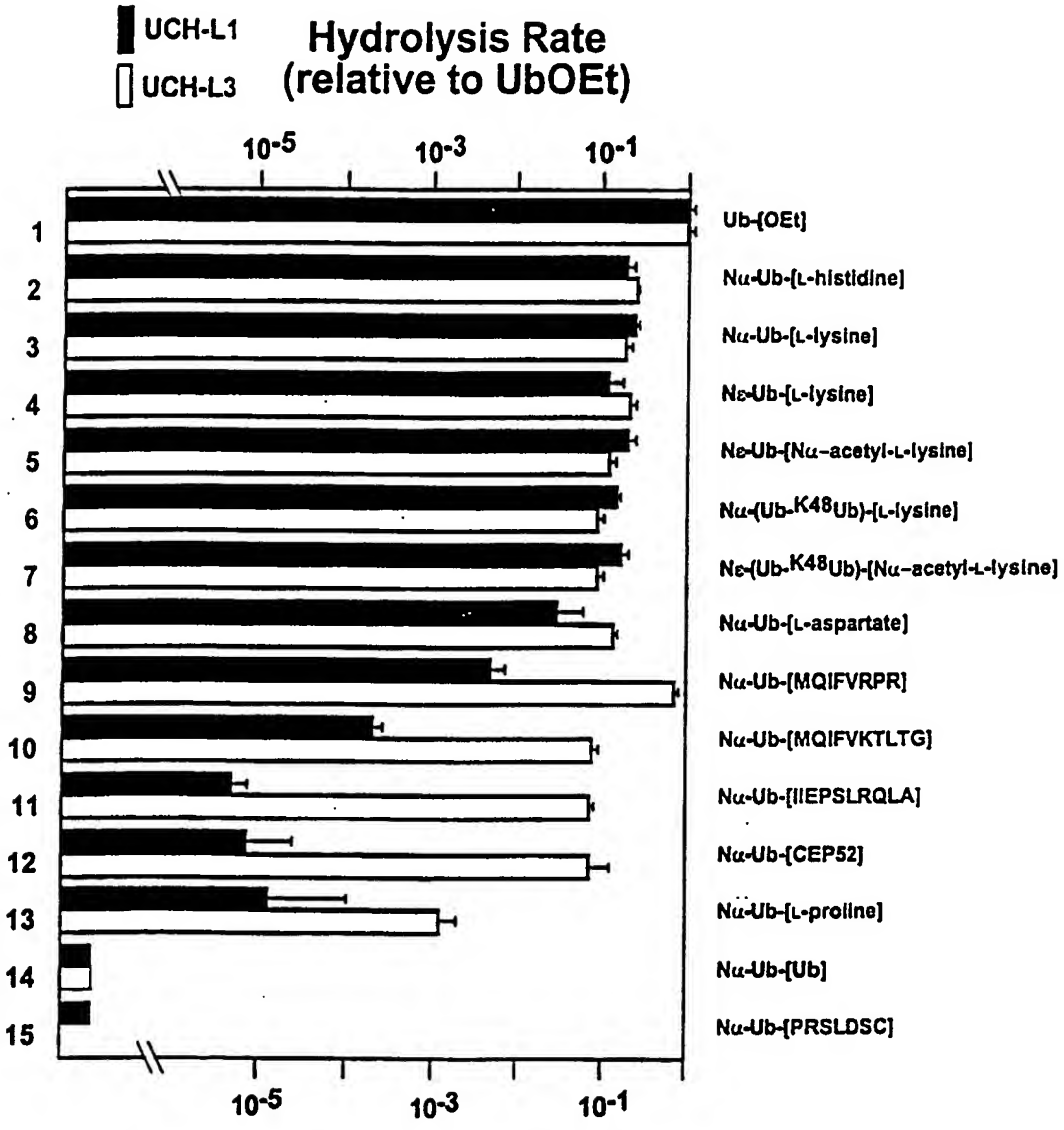
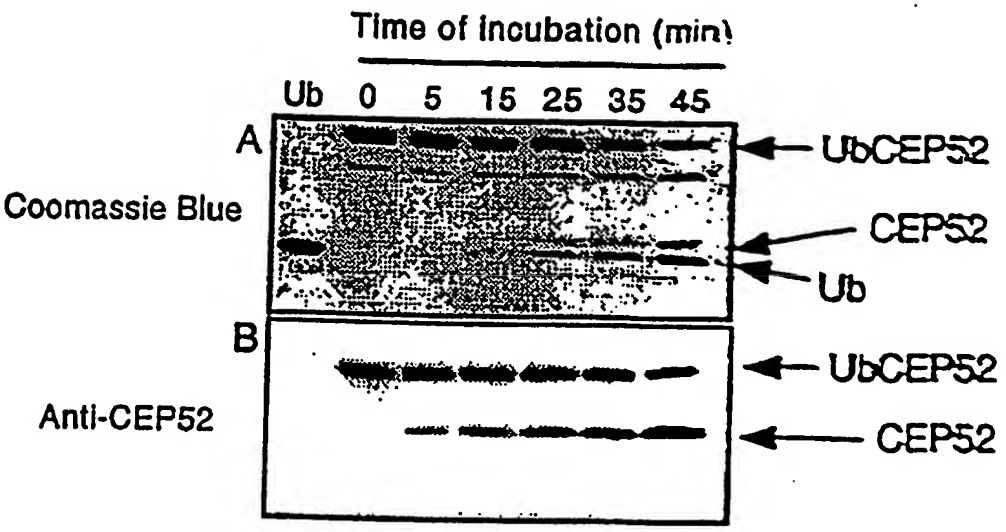

UCH-L1
UCH-L3
Hydrolysis Rate
(relative to UbOEt)
10-5
10-3
10-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Ub-[OEt]
Nα-Ub-[L-histidine]
Nα-Ub-[L-lysine]
Nε-Ub-[L-lysine]
Nε-Ub-[Nα-acetyl-L-lysine]
Nα-(Ub-K48Ub)-[L-lysine]
Nε-(Ub-K48Ub)-[Nα-acetyl-L-lysine]
Nα-Ub-[L-aspartate]
Nα-Ub-[MQIFVRPR]
Nα-Ub-[MQIFVKTLTG]
Nα-Ub-[IIEPSLRQLA]
Nα-Ub-[CEP52]
Nα-Ub-[L-proline]
Nα-Ub-[Ub]
Nα-Ub-[PRSLDSC]

FIG. 10


Time of incubation (min)
Ub 0 5 15 25 35 45
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Coomassie Blue
UbCEP52
CEP52
Ub
B
Anti-CEP52
UbCEP52
CEP52

FIG. 11

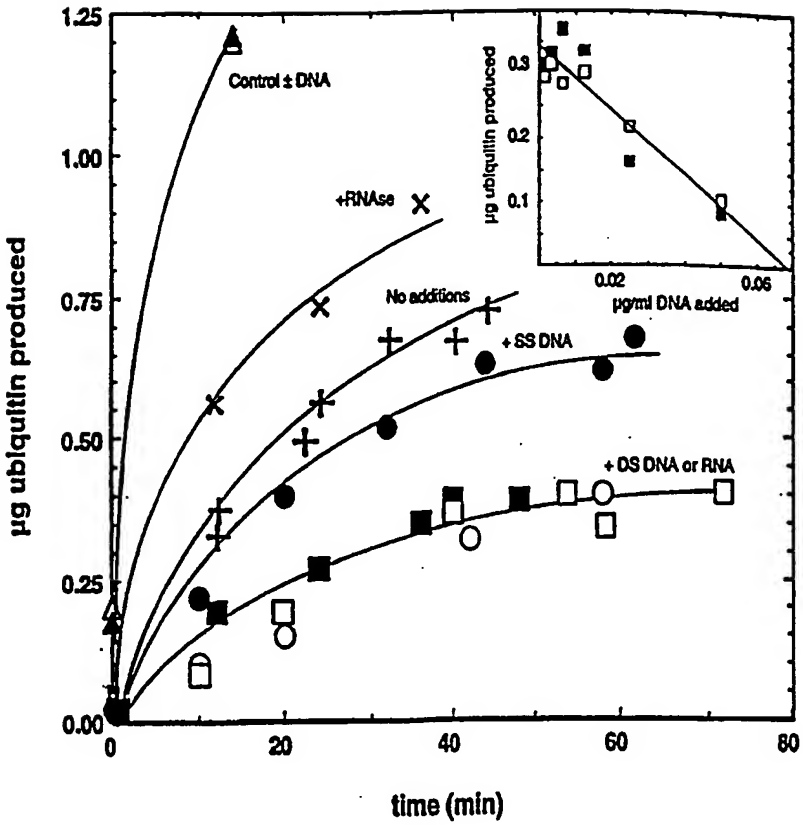

1.25
1.00
0.75
0.50
0.25
0.00
µg ubiquitin produced
0
20
40
60
80
time (min)
Control ± DNA
+RNAse
No additions
+ SS DNA
+ DS DNA or RNA
µg ubiquitin produced
0.3
0.2
0.1
0.02
0.06
µg/ml DNA added

FIG. 12

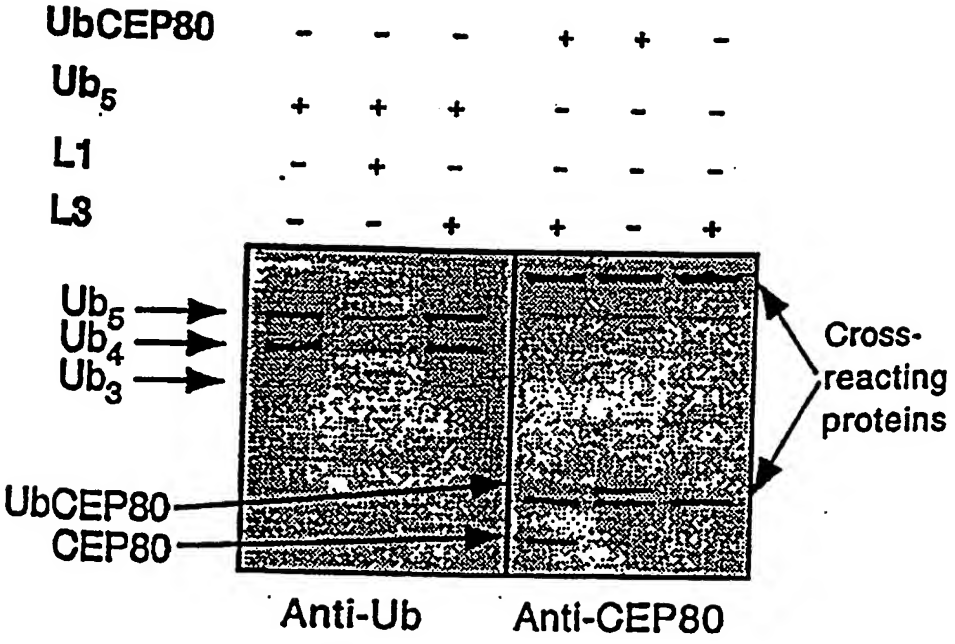

UbCEP80 - - - + + -
Ub5 + + + - - -
L1 - + - - - -
L3 - - + + - +
Ub5
Ub4
Ub3
UbCEP80
CEP80
Cross-reacting proteins
Anti-Ub
Anti-CEP80

FIG. 13

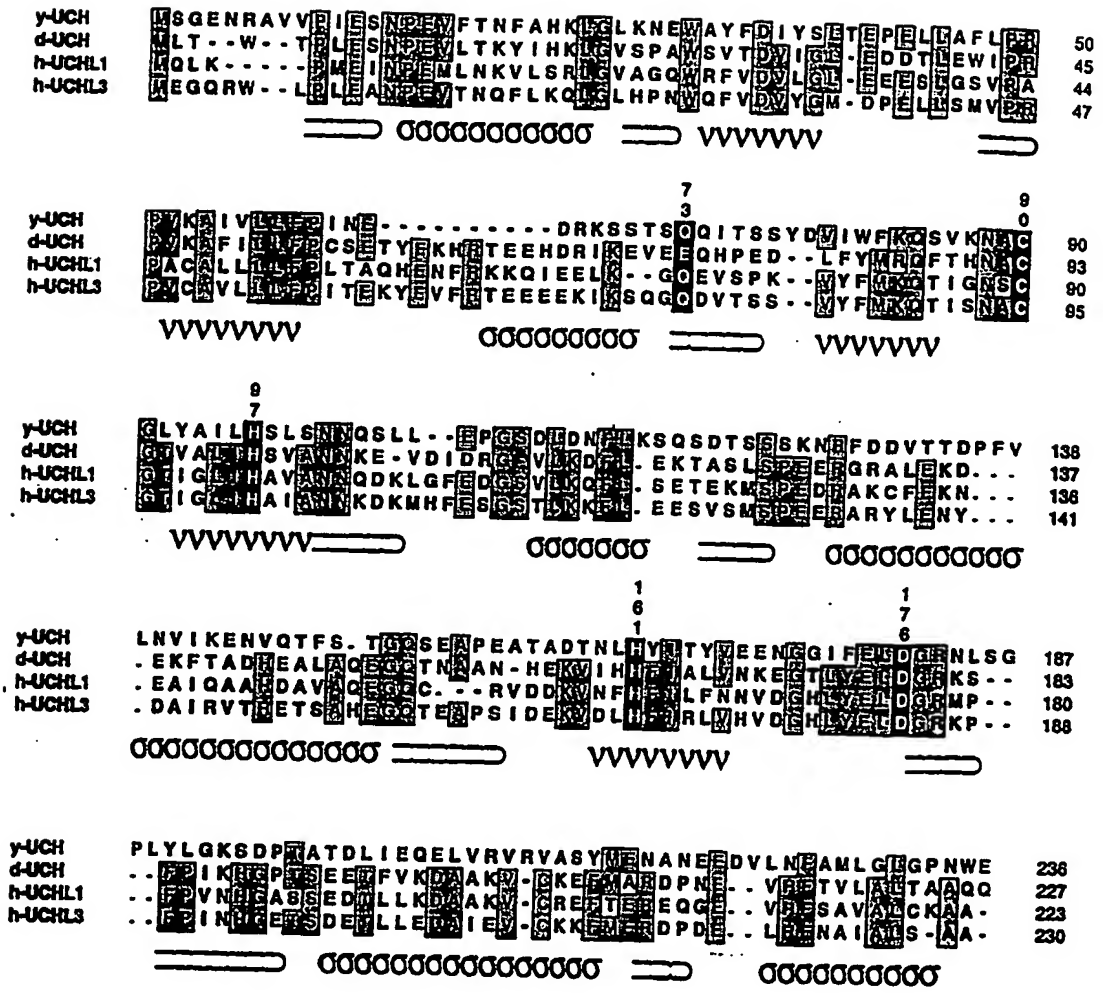


FIG. 14

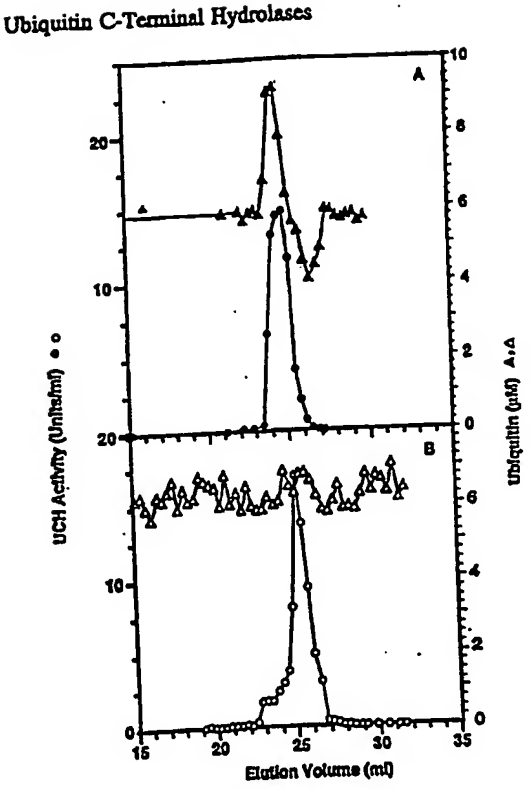
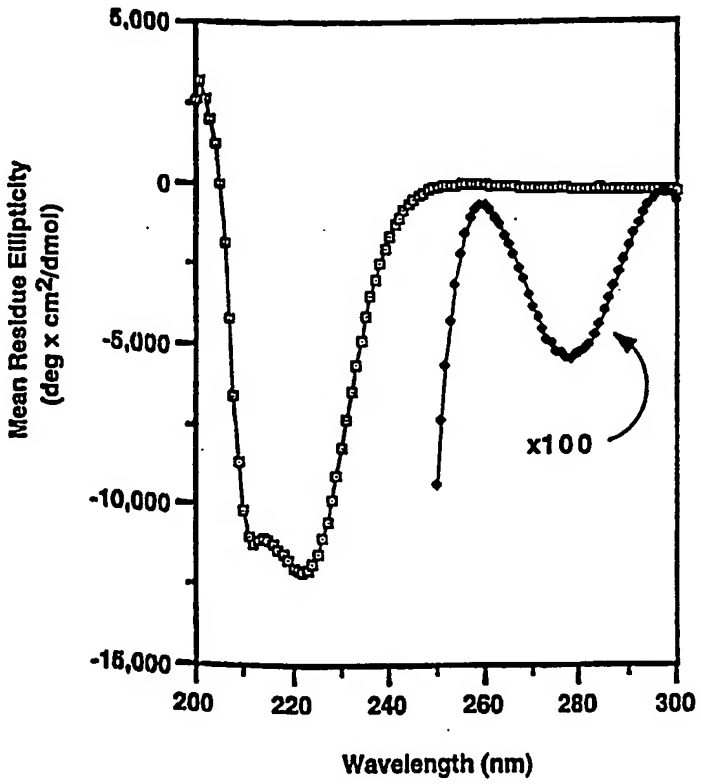

Ubiquitin C-Terminal Hydrolases
A
B
UCH Activity (Units/ml) • o
Ubiquitin (μM) ▲-▲
Elution Volume (ml)
20
10
0
15
20
25
30
35
10
8
6
4
2
0

FIG. 15A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-10,000
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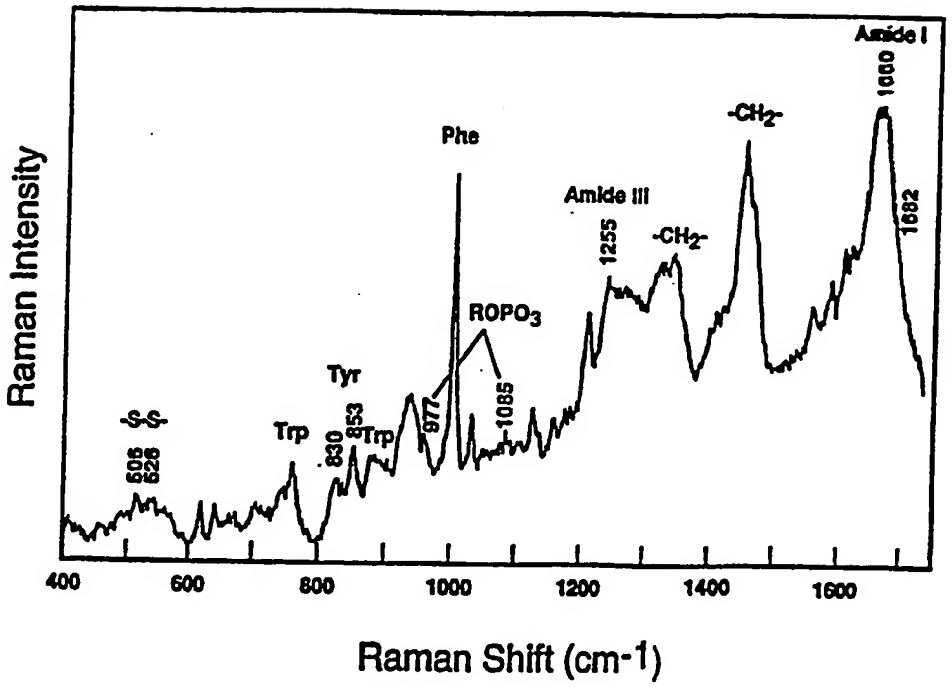
240
260
280
300
Wavelength (nm)

FIG. 15B


Raman Intensity
Raman Shift (cm-1)
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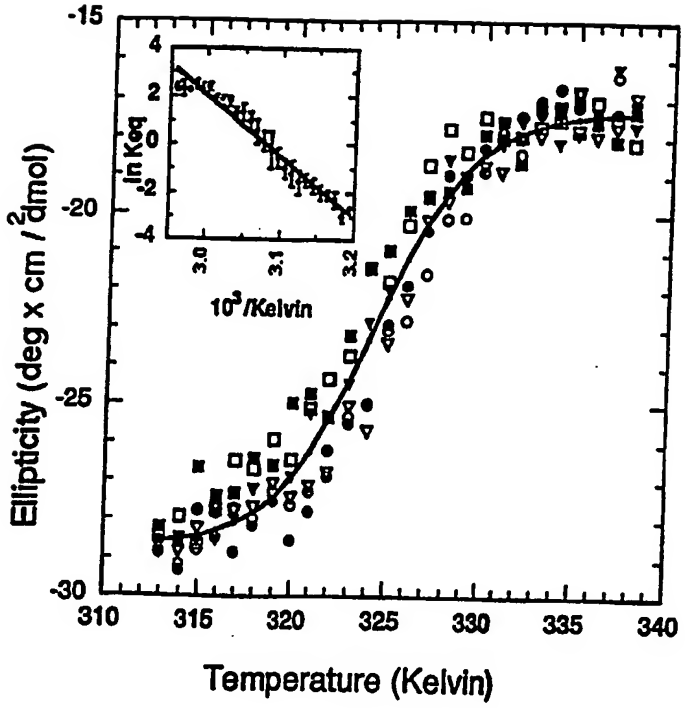
600
800
1000
1200
1400
1600
-S-S-
505
526
Trp
830
Tyr
853
Trp
977
Phe
ROPO3
1085
Amide III
1255
-CH2-
-CH2-
Amide I
1660
1682

FIG. 16


-15
-20
-25
-30
310
315
320
325
330
335
340
Ellipticity (deg x cm / dmol)
Temperature (Kelvin)
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0
-2
-4
ln Keq
3.0
3.1
3.2
10³/Kelvin

FIG. 17

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Wilkinson, Keith D.
Johnston, Steven C.
Larsen, Christopher N.
Cook, William J.

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SEQUENCE LISTING

<110> Hill, Christopher P.
Wilkinson, Keith D.
Johnston, Steven C.
Larsen, Christopher N.
Cook, William J.

<120> METHODS AND COMPOSITIONS FOR A DEUBIQUITINATING ENZYME AND VARIANTS THEREOF

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(54) Title: METHODS AND COMPOSITIONS FOR A DEUBIQUITINATING ENZYME AND VARIANTS THEREOF

(57) Abstract

The present invention relates to methods for the identification of candidate inhibitor substances that inhibit deubiquitinating activity based on the x–ray crystallographic structure of the active site of the enzyme. Changes in the properties of the enzyme are useful in identifying such substances. Also disclosed are variants of the enzyme that are useful in deubiquitinating proteins and small peptides.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13776

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/00, 1/44; C12N 9/18; G01N 33/53
US CL :435/4, 7.6, 19, 197

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 7.6, 19, 197

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and STN (Bioscience and Patents Indexes): ubiquitin C-terminal hydrolase, UCH, UCH-L3, ubiquitin, hydrolase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,585,466 A (CARTER) 17 December 1996, col. 3, lines 11-57.	1, 2, 10-12
Y,E	US 5,834,228 A (BECKER ET AL.) 10 November 1998, col. 6, lines 14-46.	1, 2, 10-12
Y	LARSEN ET AL. Substrate Binding and Catalysis by Ubiquitin C-Terminal Hydrolases: Identificaiton of Two Active Site Residues. Biochemistry. May 1996. Vol. 35. pages 6735-6744.	1, 2, 10-13, 15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
18 NOVEMBER 1998	13 JAN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LISA J. HOBBS, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13776

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1, 2, 10-13 and 15

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/US98/13776

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 2, 10-12, drawn to methods of preparing a molecule which selectively binds.
Group II, claims 3-6, drawn to methods of identification of inhibitors of enzyme activity.
Group III, claims 7-9, drawn to methods of identification of inhibitors of gene expression.
Group IV, claims 13, 15, variant UCH-L3 enzymes.
Group V, claim 14, drawn to a process of regulating deubiquitination.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the methods of Group I have the special technical feature of the binding molecule, which is not present in Groups II-V; the methods of Group II have the special technical feature of identifying enzyme inhibitors, which is not present in Groups I and II-V; the methods of Group III have the special technical feature of identifying gene expression inhibitors, which is not present in Groups I-II and IV-V; the product of Group IV has the special technical feature of the enzyme, which is not present in Groups I-III and V; the process of Group V has the special technical feature of regulating deubiquitination, which is not present in Groups I-IV.